



Efficacy of Chloroquine in Reducing Post-Orthodontic Relapse (An Animal Model Study)

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Abstract

RETAINING teeth in their proper alignment after orthodontic treatment can be highly challenging. This study aimed to estimate the effectiveness of locally injected chloroquine in improving bone remodeling and reducing post-orthodontic relapse. The third incisors on the right and left lower quadrants of six sheep were removed, and the second and fourth incisors were brought close together by using a sectional orthodontic appliance. Chloroquine solution was injected near and parallel to the mesial surface of the second incisor and the distal surface of the fourth incisor on one quadrant while the other quadrant received 1x phosphate buffered saline as a control. Four weeks later, the orthodontic appliance was debonded and the teeth were allowed to move back. By utilizing digital analysis, the relapse distance of the approximated incisors was measured at 21 and 42 days respectively after appliance removal. Histological and mRNA expression analysis of osteogenic marker (Runx2) were conducted to evaluate the periodontal space width and the new alveolar bone formation of the chloroquine-injected and control sides incisors. clinical and histological measurements showed that the approximated incisors in the chloroquine injected quadrant had a significantly shorter relapse distance ($p \leq 0.05$), significantly smaller periodontal ligament width ($p \leq 0.05$) and significantly larger new bone area formation ($p \leq 0.05$) than in the control quadrant. The mRNA expression level of the osteogenic marker (Runx2) was significantly larger ($p \leq 0.05$) in the chloroquine-injected quadrant than in the control quadrant. The outcomes of this research suggest that chloroquine can enhance bone remodeling and reduce post-orthodontic relapse in sheep.

Keyword: Chloroquine, Orthodontic Relapse, Runx2, qRT-PCR, Sheep.

Introduction

Orthodontic treatment aims to correct malocclusions and achieve stable occlusion and alignment of the teeth. However, maintaining the achieved tooth positions over the long term, known as the retention phase. Retaining teeth in the new satisfactory position after orthodontic correction is one of the most difficult tasks in orthodontics. The success of orthodontic treatment depends heavily on effective retention protocols to prevent relapse [1].

The etiology of relapse in orthodontics is complex and unclear and involves several factors that compromise the stability of the results, such as the time of gingival and periodontal tissue reorganization, unstable position of the teeth after orthodontic treatment, retention technique, patient compliance, age, ultimate occlusion following treatment and changes produced by growth [2–4].

Information from histological and molecular studies has suggested that the removal of orthodontic appliances might elicit immediate changes in the mechanical conditions, which could result in alterations resembling those encountered during active treatment [5] Under the control of biochemical networks similar to those stimulating orthodontic tooth movement, the osteoclasts redistribute in the direction of relapse, while bone is formed on the opposite side [6–8].

As orthodontic tooth movement can be modulated by any substance administered that can have effects on the molecular pathways involved, [9,10] the potential impact of different medications on the rate of orthodontic tooth movement has been systematically reviewed previously, and various effects have been shown in animals [11–13]. Effects in human subjects have also been documented [14].

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Chloroquine, anti-malarial, anti-inflammatory and autophagy/lysosome inhibitor used to treat rheumatoid arthritis, pulmonary hypertension and systemic lupus erythematosus reduces RANKL-induced (Receptor activator of NF- κ B ligand) osteoclast formation and function by increasing TRAF3 (TNF related factor) expression in osteoclast precursors in vitro and in vivo [15].

To the best of our knowledge, no previous study has investigated the efficacy of the local application of chloroquine in reducing post-orthodontic relapse. Thus, the principal objectives of this study were to evaluate clinically, histologically and on the level of molecular biology the effectiveness of chloroquine in improving bone remodelling and hence, reducing the relapse of orthodontically moved teeth. So the null hypothesis of this study that chloroquine had no effect on bone remodelling and on post orthodontic relapse.

Material and Methods

Study setting

The study was conducted in the animal household of the College of Veterinary Medicine/University of Mosul. The University Mosul Dental School's research ethics committee reviewed and endorsed the research protocol (Approval No. UoM. Dent. 23/18 on 02.05.2023). A comprehensive examination was carried out by veterinarians on Awassi sheep to assess their general, periodontal, and dental health. The sheep were quarantined for 2 weeks before the beginning of the experiment. The following formula was performed to calculate the sample size:

$$(n = DF/k + 1) \quad (16)$$

Where: n = number of subjects per group, DF = the between-subject error (that is, the within-subject DF) based on the acceptable range was set to 10 and k = number of groups and equal to 2. The final sample size was calculated as the sample size per group and equal to 6 (16).

Study design

The study comprised six adult male sheep, all of which were 55 kg on average and had a mean age of three years. Each sheep possesses a total of eight permanent incisors, with four located on each side. To determine the effects of chloroquine, a prospective randomized split-mouth experimental trial was conducted. The right and left sides of the sheep were randomly assigned to either the chloroquine group (CHG) or the control group (CG) based on a digitally produced sequence of random numbers. The study involved the second and fourth incisors on each side, and the third incisor on each side was removed. Throughout the intended experiment, the sheep were anaesthetized numerous times with ketamine (22 mg/kg intramuscularly) and xylazine (0.2 mg/kg intramuscularly). Each sheep

had its third incisors removed on both the right and left sides, and the areas were permitted to heal for a week.

The sheep were anaesthetized, and the labial surfaces of the second and fourth incisors on each quadrant were polished using pumice powder (Produits Dentaires SA., Switzerland) and a dental polishing brush using low-speed handpiece (Coxo Medical Instrument CO., LTD., China). Then, teeth surfaces were etched with phosphoric acid 38% (Pulpdent Co., USA) for thirty seconds, washed with water for fifteen seconds, and air dried. Standard 0.022 x 0.028 inch slot edgewise metal brackets of Equilibrium[®] 2 series (Dentaurum GmbH & Co., Germany) were bonded using TrueBond LC (IOS Corp., USA) and cured by Eighteenth Curing Pen E (Changzhou Sifary Medical Technology Co., China) for twenty seconds from each direction. Each bracket was attached at the midpoint of the tooth's long axis, in a mesiodistal direction. The brackets were all of equal height by using bracket positioning gauge (Dentaurum GmbH & Co., Germany) to ensure there were no differences in vertical alignment among the bonded incisors. The two incisors on either side were then joined together using sectional 0.017x 0.025 inch stainless steel orthodontic arch wire, and their ends were bent to make a non-traumatic end. One loop of elastomeric chain (Orthometric, Marília, Brazil) was pre stretched and applied to the second and fourth incisors to achieve a starting force of 150 grams. Three times per week, the elastomeric chain was replaced until the second and fourth incisors had completely approximated. A reciprocal anchorage was developed in this mechanics. Then the brackets on the bodily moved approximated teeth were passively ligated using stainless steel ligature wire 0.010 inch. (Dentaurum GmbH & Co., Germany) for four weeks (Fig.1). The total time lapsed from the beginning of the procedure till the slaughtering of the last group was fifteen weeks.

Chloroquine was prepared for injection by dissolving 10 mg Chloroquine powder (Chem-Impex INTL INC., USA) in 1 millilitre of 1x phosphate buffered saline (Chem-Impex INTL INC., USA) solution [17].

A chloroquine injection was administered to a randomly selected side of each animal utilizing a disposable one-unit insulin syringe equipped with a 25-gauge microneedle. (Forlong Medical Corp., China). The injection was given adjacent and parallel to the mesial surface of the second incisor and the distal surface of the fourth incisor at the mucogingival junction, penetrating through the attached gingiva into the oral mucosa. The dose was divided, with 0.5 ml injected into the labial side and another 0.5 ml injected into the lingual side of the vestibular mucosa. This technique replicates the method of infiltrative local anaesthetic injections described in a study conducted by [18–21].

A volume of 1 millilitre of 1x phosphate buffered saline solution was injected on the opposite side using the same injection technique, serving as a control. The injection was performed once a week for four weeks.

Clinical Assessment

After completing the injection procedure, the fixed orthodontic device was debonded utilizing bracket removal pliers (Dentaurum., Germany) and an intraoral impression Zhermack Zetaplus A Silicone impression Material (Badia Polesine (RO), Italy) loaded into a customized resin tray will be obtained immediately after the orthodontic appliances removal and 21, 42 days later of post-orthodontic relapse to create dental models. All impressions were poured with improved die stone (Elite Rock Dental Stone; Zhermack, Badia Polesine, Italy). All stone models were scanned with an E1 lab scanner (3Shape Co., USA) to generate three-dimensional models in stl. file format. Utilizing the Viewbox software (V 4.0.1.7; dHal Software, Greece), The models were aligned according to the mandibular occlusal plane, which was determined by the position of the incisal edge of the lower incisors. Two line were constructed at right angles to the occlusal plane of the mandible. The first line was initially sketched on the distal surface of the second incisor, precisely at the location of the furthest contact region. A second line was sketched to make contact with the most mesial contact area of the mesial surface of the fourth incisor. The post-orthodontic relapse is quantified by measuring the linear distance, which runs parallel to the mandibular occlusal reference plane, between the two constructed vertical lines. Measurements were obtained utilizing the ruler tool in the Viewbox software, Figs(2,3). This method is similar to that proposed by ALSwafeeri et al [22].

The measurements were conducted by the main investigator in a blinded fashion. To enhance the level of confidence, the intra-examiner errors pertaining to post-orthodontic relapse measurements were evaluated by having the same investigator replicate the assessments of five randomly selected three-dimensional models two weeks apart. The co-investigator repeated measurements on five randomly selected three-dimensional models in order to identify any inter-examiner errors.

On 21 and 42 days of orthodontic appliance removal 3 sheep were slaughtered, and a dense diamond saw (Cadence Inc., USA) was utilized to precisely cut the anterior portion of each mandible 5 mm distal to the fourth incisor. A sterile 4-mm diameter trephine drill (NTI-Kahla GmbH, Germany) using a low-speed handpiece (COXO Medical Instrument Co., Ltd., China) and profuse Sodium Chloride 0.9% Irrigation Solution (Jedux Parenteral Private Limited, India) was employed to remove bone and root tissue from the distal aspect of the fourth incisor of all samples parallel to the visible inclination of the fourth incisor root through full thickness of the alveolar bone. Then, the removed tissues were placed in sterile micro-centrifuge tubes containing 1.5 ml of DNA/RNA shield lysis solution

(Zymoresearch, Irvine, USA) and stored at -20° C for further RNA extraction and qRT-PCR analysis

Histological assessment

The specimens were fixed for three days in a 10% neutral buffered formalin solution, followed by decalcification for five to six weeks in an 8% hydrochloric and 8% formic acid solution. Then, by using a surgical blade, the second incisor on each side was cut into a separate block for paraffin wax embedding. Utilizing a standard laboratory protocol, serial longitudinal sections five micrometres thick were prepared and subjected to staining with hematoxylin and eosin. An optical microscope AmScope SM-3T (United Scope LLC., USA) was utilized for histological investigation of the periodontal space of each second incisor longitudinally from the alveolar bone crest to the root apex. Images of selected regions were captured using an 8-megapixel digital camera mounted to the microscope. These pictures were utilized to perform histomorphometric analyses. The image scale calibration was performed using Image J software V1.54h. A square grid was placed on top of the histologic sections. Each side of the square grid measured 1 mm. In order to assess the impact of melatonin on the periodontium, certain parameters were examined. This included periodontal ligament width and areas of new bone formation. For the measurement of periodontal ligament width, the width from the mesial surface of the lower mandibular second incisor root to the alveolar bone was measured under a digital microscope with 50x magnification. The root is divided into cervical and apical half and 5 measurements were made in each half.

RNA extraction and cDNA synthesis

Samples in micro-centrifuge tubes were crushed using a pestle and mortar and subjected to ultrasonic cell disruption using Vibra-Cell 500 processor (Sonic & Materials, Inc., Newtown, USA). RNA was extracted from the homogenate according to the manufacturer's protocol using AddPrep Total RNA Extraction Kit (Add Bio Inc., Yuseong-gu, South Korea). Then, the extracted RNA concentration was quantified spectrometrically using a Nanodrop 2000 (ThermoFisher Scientific Inc., USA). 2 µl of extracted RNA was used for the synthesis of complementary DNA (cDNA) using AddScript cDNA Synthesis Kit (Add Bio Inc., Yuseong-gu, South Korea). Thermal cycler steps of cDNA Reverse Transcription were 25 °C for five minutes, then 42 °C for thirty minutes and lastly 5 °C for five minutes. The resultant cDNA was stored at -20°C until further qRT-PCR was carried out.

Gene selection and primer sequence

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as the housekeeping gene and

Runt-related transcription factor 2 (RUNX2) as the target gene. The primer sequence of the reference gene had been previously published by [23] and of the target gene by [24] (Table 1).

On 21 and 42 days of orthodontic appliance removal 3 sheep were slaughtered, and a dense diamond saw (Cadence Inc., USA) was utilized to precisely cut the anterior portion of each mandible 5 mm distal to the fourth incisor. A sterile 4-mm diameter trephine drill (NTI-Kahla GmbH, Germany) using a low-speed handpiece (COXO Medical Instrument Co., Ltd., China) and profuse Sodium Chloride 0.9% Irrigation Solution (Jedux Parenteral Private Limited, India) was employed to remove bone and root tissue from the distal aspect of the fourth incisor of all samples parallel to the visible inclination of the fourth incisor root through full thickness of the alveolar bone. Then, the removed tissues were placed in sterile micro-centrifuge tubes containing 1.5 ml of DNA/RNA shield lysis solution (Zymoresearch, Irvine, USA) and stored at -20°C for further RNA extraction and qRT-PCR analysis

Gene selection and primer sequence

Quantitative Real-Time Reverse Transcriptase–Polymerase Chain Reaction (qRT-PCR)

The mRNA expression levels of the reference and target genes were determined using Sybr green quantitative reverse transcriptase polymerase chain reaction (qRT PCR) assays. A master mix for 12 samples was prepared for each of the reference and target genes. The reactions were run in duplicate using 10 μL SYBR Green (Add Bio Inc., Yuseong-gu, South Korea) 1 μL forward primer, 1 μL reverse primer, 5 μL distilled water and 3 μL cDNA with a total volume of 20 μL . The DNA amplification was executed using the PowerAmp96™ DX Real-Time PCR system (Kogenebiotech, Seoul, Korea). The cycling protocol was programmed according to the thermal profile Shown in Table (2) for GAPDH and RUNX2 genes.

The confirmation of amplification specificity was achieved through the analysis of the melting curve. The mRNA level of the RUNX2 gene was determined by measuring the threshold cycle (Ct) value as a relative level to that of GAPDH through the equation $2^{-\Delta\text{Ct}}$ ($\Delta\text{Ct} = \text{RUNX2 Ct} - \text{GAPDH Ct}$). As the Ct value increases, the level of gene expression decreases. The $2^{-\Delta\Delta\text{Ct}}$ method (Livak method) was used for relative quantification of RUNX2 gene expression [25]. The acquired result was a fold increase or decrease of the RUNX2 gene mRNA expression level in the test samples relative to the calibrator sample (normal sample).

Statistical analysis

Statistical analysis was conducted using SPSS V26 (Statistical Package for Social Science, IBM Inc., USA). The data were tested for their normal

distribution by using the Shapiro-Walk test and all data were normally distributed. Comparison between the Chloroquine group (CHG) and Control group (CG) regarding relapse distance, periodontal ligament width and new bone formation area was done on 21 and 42 days respectively after removal of the orthodontic appliance using independent sample t-test or Mann Whitney U test depending on a normal distribution of data. A significance level of 0.05 (two-tailed) was used to determine statistical significance for all analyses.

Results

Relapse Distance and Relapse Percentage

Based on millimetric measurements obtained from digital analysis of 3-dimensional models, significantly more relapse was observed in CG than CHG on 21 and 42 days after appliance removal (Table 3).

Periodontal ligament width and New bone formation

Histological measurement revealed a substantial difference in the periodontal ligament width mean between CHG and CG at 21 and 42 days after appliance removal, respectively (Table 4).

Microscopical findings in the alveolar bone adjacent to the mesial surface of the second incisor root of CHG at 21 and 42 days after appliance removal revealed outstanding and variable observations. Newly produced bone, both mature and immature, with several groups of osteoblasts, was seen along the boundary of the alveolar socket and within the periodontal space, delineating the remodelled region of the socket wall. Several bony spicules with newly produced blood vessels were also seen adjacent to the bone surface (Figure 4).

Based on the histological measurement of the new bone formation area a significant difference in the mean was observed between MG and CG at 21 and 42 days respectively after appliance removal (Table 5).

RUNX-2 mRNA expression analysis

The RUNX-2 mRNA expression level was inclined to up-regulate continuously with a maximum 12.20-fold change at 42 days and 11.56-fold change at 21 days after appliance removal in CHG figure (5) which was significantly higher ($p \leq 0.05$) than CG (Table 6).

Discussion

The present study employed the sheep as studying model because of the morphological and periodontal resemblance between its lower incisors and human incisors. In addition, It has been shown that the bucco-lingual alveolar bone resorption in a sheep following dental extraction is comparable to the healing pattern in humans. The macrostructure

of ovine bone is very similar to human bone, and bone composition and remodelling are moderately similar [26].

In order to minimize inter-subject variability for potential confounding variables including oral hygiene, dental movement, and body weight, a split mouth design was adopted in this study. To the best of our knowledge this study is the first to assess the efficacy of Chloroquine solution injection in reducing post-orthodontic relapse.

The Chloroquine solution was injected submucosally, a mode of drug delivery that is widely regarded as safe, suitable and clinically acceptable by orthodontic patients [19]. The injected Chloroquine was effective in decreasing post orthodontic relapse as shown by 3-dimensional model, histological and osteogenic marker expression analysis.

Based on 3-dimensional model analysis more pronounced relapse was observed in CHG than in GC and this was in agreement with Hassan et al., [27] who injected Dried bone matrix protein in the periodontal space of ovine incisors. Furthermore, this result agreed with a result obtained by Tokhtah & Alhadlaq [28] as they injected bisphosphonate gel in the periodontal space of goats incisors.

Histological observations of this study regarding newly formed bone area extending from alveolar socket wall toward root cementum minimizing the width of periodontal space of relapse pressure side of the orthodontically moved incisors in CHG could be the main reason for decreasing post orthodontic relapse. Additionally, active bone remodelling, as observed by the presence of osteoblasts and osteoclasts around the newly produced bone islands in the periodontal ligament space in the CHG is in accordance with a study conducted by Hassan et al., and Tokhtah & Alhadlaq [27,28].

In terms of the activity of bone-resorbing osteoclasts, the secretion of lysosomal vesicles containing protons and matrix-degrading proteinases into the resorption lacunae is essential. The lysosomal secretory vesicles in osteoclasts contain protons for decalcifying the mineralized matrix and enzymes for digesting organic matrix. Such lysosomal enzymes are activated at a low pH, acidification of the lysosome is required for the degradation of bone matrix proteins [29]. Chloroquine, an anti-malarial chemical, is an autophagic inhibitor which blocks autophagosome fusion with lysosome and slows down lysosomal acidification [30]. Thus, the result of this research is in agreement with the findings of Al-Bari et al., study on mice who concluded that Chloroquine suppressed the bone resorbing activity of osteoclasts by inhibition of the acidification in the lysosomes, as well as osteoclast differentiation [29]. So the

chloroquine has a bone-increasing effect by inhibiting osteoclast differentiation and function.

In the present study, the mRNA level of RUNX-2 a typical pro-osteogenic factors was highly expressed in CHG as compared to CG and this was in consistence with a study conducted by Cai *et al.*, who observed that RUNX-2 were significantly upregulated when vascular smooth muscle cells were cultured in calcification medium. However, this effect was markedly enhanced by chloroquine treatment. Chloroquine treatment enhanced calcification and osteogenic transformation in cultured vascular smooth muscle cells [31].

The result of a recent research conducted by He *et al.*, support the findings of our study as they observed that pharmacological inhibition of autophagy, via chloroquine, may reduce inflammation, osteoclastogenesis, and bone resorption in experimental periodontitis with excessive autophagy level [32].

Another recent study conducted by Mahmoud et al., affirmed the results of our study as they noted that chloroquine showed valuable anti-osteoporotic effects which can be attributed to its inhibitory effects on autophagy-lysosomal pathway thus inhibiting osteoclast differentiation. Furthermore; it inhibited ERK protein kinases activation with subsequent decrease in receptor activator of nuclear factor- κ B ligand RANKL expression on osteoblastic cells. Besides, chloroquine restored serum osteoprotegrin which in turn increased expression of osteoprotegrin on osteoblastic cells and promotes bone formation [17].

On the other side, the digital analysis of three-dimensional models revealed that the moved incisors of the CG experienced a more pronounced relapse at 21 and 42 days, respectively, after the removal of the orthodontic appliance than the incisors of CHG. Histological examinations revealed increased periodontal ligament width and areas of osteoclastic activity, indicative of a substantial relapse that occurred.

Nevertheless, there were some limitations in this study such study duration and one concentration of chloroquine used, It is recommended to administer greater dosages of the medication in future research and compare them with the current concentration to establish the most effective dose of chloroquine for reducing post-orthodontic relapse.

Conclusion

Within the limitation of this study, our preliminary results indicate that the null hypothesis of this study is rejected and the local application of chloroquine improves bone remodelling by interfering with equilibrium of osteoblastogenesis and osteoclastogenesis cycle favoring osteoblastogenesis and promotion of new bone

formation, Thus potentially reduce post-orthodontic relapse in sheep and it has the potential to be part of the biomodulation protocol of orthodontic relapse control.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgement

Not applicable

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TABLE 1. Primer Sequences for housekeeping gene (GAPDH) and target gene (RUNX2)

Gene	Forward	Reverse
GAPDH	5'-ACAGTCAAGGCAGAGAACGG-3'	5'-CCAGCATCACCCCACTTGAT-3'
RUNX2	5'-TTCGCCTCACAAACAACCAC-3'	5'-GTGCTCGGATCCCAAAAGAA3'

TABLE 2. Thermal profile of GAPDH and RUNX2 mRNA expression.

Step	Temperature	Duration	Cycles
Enzyme activation	50°C	2 min	Hold
Denaturation	95°C	10 min	40
Annealing	94°C	15 sec	
extension	60 °C	1 min	

TABLE 3. Comparison of orthodontic relapse between studied groups.

Groups	Time (day)	Relapse distance		p value
		mm	%	
CHG (n=6)	21	2.59±0.28	42.60	0.048
CG (n=6)		3.38±0.39*	55.59	
CHG (n=3)	42	2.75±0.11	45.23	0.000
CG (n=3)		4.13±0.11*	67.93	

Data expressed as Mean±SD, *indicate significantly higher at $p \leq 0.05$ using independent sample t test, CHG= Chloroquine group, GC=control group, mm= millimeter

TABLE 4. Comparison of Periodontal ligament width between studied groups.

Groups	Time (day)	Width (μm)	p value
CHG (n=3)	21	524.8±79.2	0.000
CG (n=3)		771.4±82.7*	
CHG (n=3)	42	458.4±58.1	0.0001
CG (n=3)		687.7±56.4*	

Data expressed as Mean±SD, *indicate significantly higher at $p \leq 0.05$ using independent sample t test, CHG=Chloroquine group, GC=control group, μm = micrometer

TABLE 5. Comparison of new bone formation area between studied groups.

Groups	Time (days)	Area (μm^2) (Mean±SD)	p value
CHG (n=3)	21	69929.3± 5117.8*	0.0001
CG (n=3)		30734.6± 5199.2	
CHG (n=3)	42	297118.8± 5967.7*	0.0001
CG (n=3)		87054.6± 4406.9	

Data expressed as Mean±SD, *indicate significantly higher at $p \leq 0.05$ using independent sample t-test, CHG=Chloroquine group, GC=control group, μm^2 = square micrometer

TABLE 6. RUNX-2 mRNA expression level and Fold change of studied groups.

Groups	Time	Mean Ct Runx2	Mean Ct GAPDH	Mean Δ Ct study sample	Mean Δ Ct Calibrator sample	Fold change of Runx2 gene expression	RUNX-2 mRNA expression ($2^{-\Delta Ct}$)	p value
CHG	21	25.20	23.43	1.76	4.01	4.76	0.29*	0.05
CG	21	24.85	22.20	2.65	4.01	2.58	0.16	
CHG	42	26.59	25.30	1.28	4.01	6.63	0.41*	0.05
CG	42	27.23	25.05	2.18	4.01	3.57	0.22	

*Indicate significantly higher at $p \leq 0.05$ using independent sample t test, CHG=chloroquine group, GC=control group, Ct=cycle threshold.

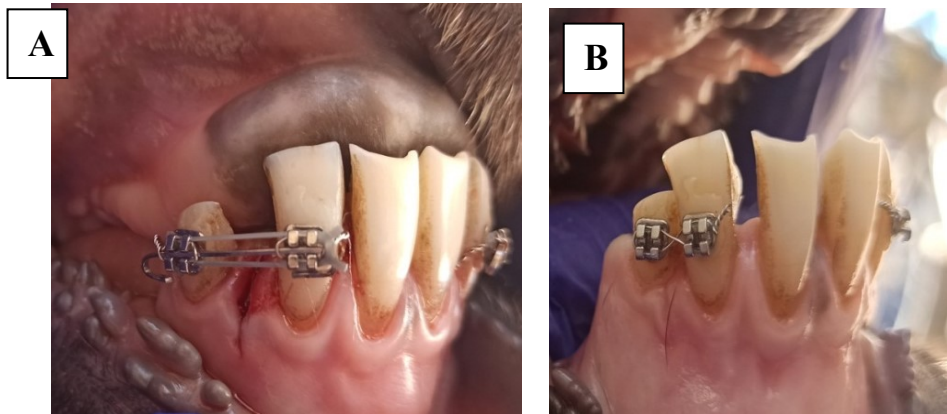


Fig. 1. (A) Post-extraction space of third incisor and orthodontic appliance in situ. (B) post-extraction space closure and passive ligation of brackets on the second and fourth incisors with stainless steel ligature wire.

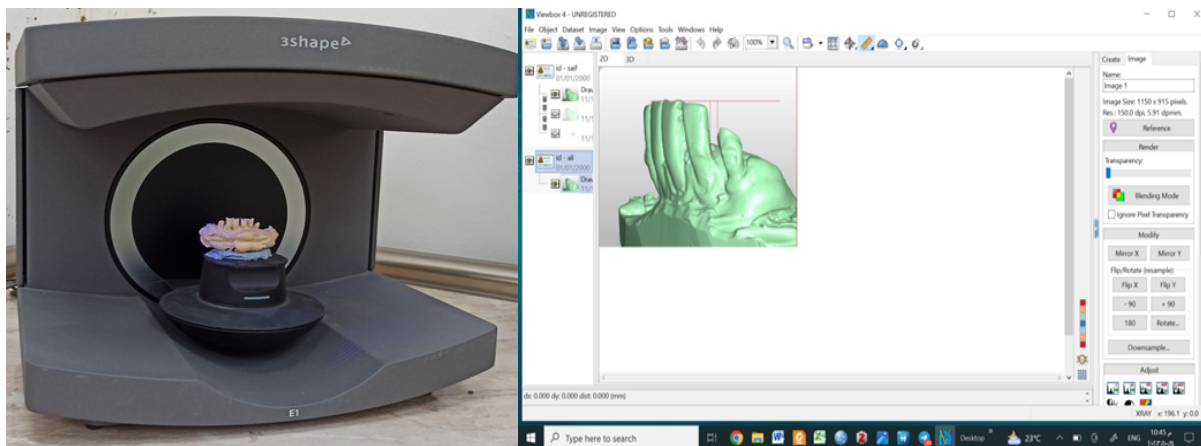


Fig. 2. Three-dimensional scanning of study models using E1 3shape lab scanner (3Shape co., USA) and measuring the magnitudes of post-orthodontic relapse using Viewbox software (dHal Software, Greece).

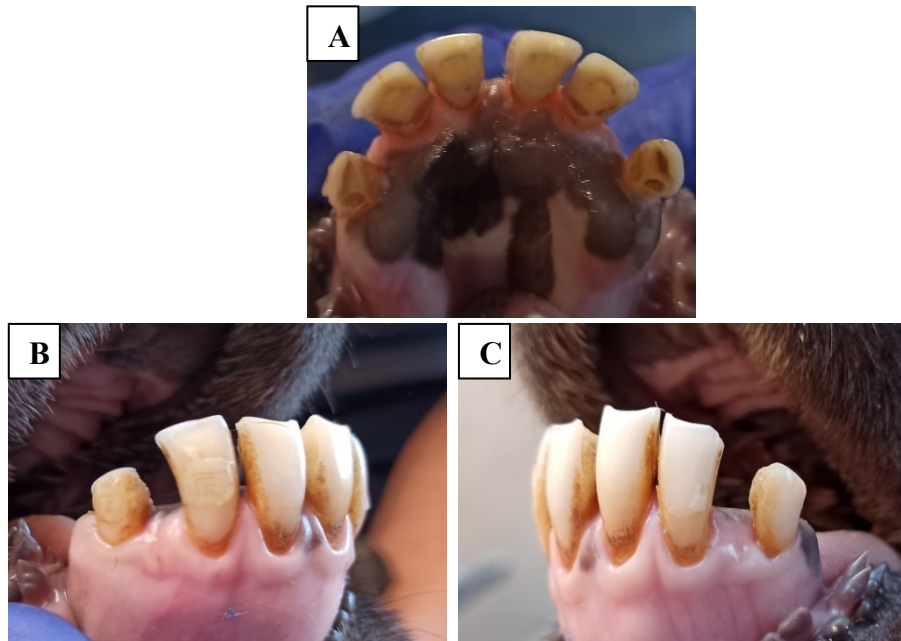


Fig. 3. (A) Occlusal view of incisors teeth 42 days after appliance removal. (B) Lateral view of incisors of chloroquine injection side. (C) Lateral view of incisors of phosphate buffered saline injection side.

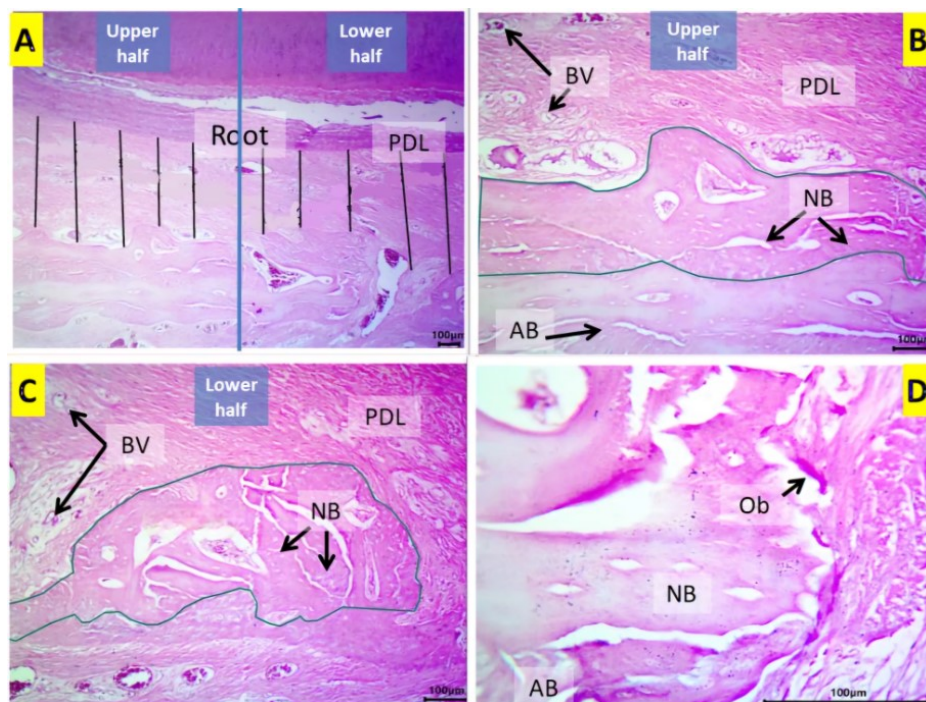


Fig. 4. Histological section of the second incisor root and the surrounding bony socket of the MG at 42 days after orthodontic appliance removal showing (A) periodontal ligament width measurement, (B) upper half (apical area), (C) lower half (cervical area) and (D) New bone formation (NB) with clusters of osteoblast (Ob) and osteoclast (Oc). Periodontal ligament (PDL), Blood vessels (BV), and Alveolar bone (AB). H&E stain, (A: 100X; B: 100X; C: 400X), Scale bar=100 μ m.

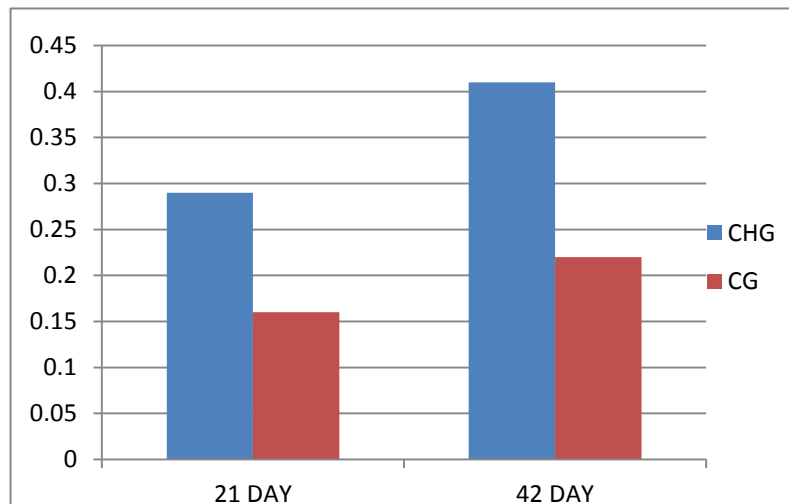


Fig. 5. RUNX-2 mRNA expression level ($2^{-\Delta Ct}$) of study groups at 21 and 42 day after appliance removal, CHG=chloroquine group, CG=control group

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تأثير دواء الكلوروكوين على مستوى عامل التئام (RUNX2) وانتكاس الاسنان بعد علاج تقويم الاسنان (دراسة نموذجية على الحيوان)

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الملخص

ان الحفاظ على الأسنان في محاذاة صحيحة بعد علاج تقويم الأسنان أمراً صعباً للغاية. هدفت هذه الدراسة إلى تقدير مدى فعالية حقن الكلوروكوين موضعياً في تحسين إعادة تشكيل العظم والحد من الانتكاس بعد تقويم الأسنان. تمت إزالة القواطع الثالثة الموجودة على الأرباع السفلية اليمنى واليسرى لستة أغنام، وتم تقريب القواطع الثانية والرابعة من بعضها البعض باستخدام جهاز تقويم الأسنان المقطعي. تم حقن محلول الكلوروكوين بالقرب وبالتوازي مع السطح الأنسي للقاطعة الثانية والسطح البعيد للقاطعة الرابعة في ربع واحد بينما تلقى الربع الآخر x1 محلول ملحي من الفوسفات كعنصر محايد. وبعد أربعة أسابيع، تم فك جهاز تقويم الأسنان والسماح للأسنان بالتحرك إلى الخلف. باستخدام التحليل الرقمي، تم قياس مسافة الانتكاس للقواطع التقريبية عند 21 و42 يوماً على التوالي بعد إزالة الجهاز. تم إجراء التحليل النسيجي و mRNA للعلامة العظمية (Runx2) لتقييم عرض مساحة اللثة والعظم السنخي المحيط بالقواطع الجانبية المحقونة بالكلوروكوين. أظهرت القياسات السريرية والنسجية أن القواطع التقريبية في الربع المحقون بالكلوروكوين كانت لها مسافة انتكاس أقصر بكثير ($p < 0.05$)، وعرض أربعة أربطة اللثة أصغر بكثير ($p < 0.05$) وتكوين منطقة عظمية جديدة أكبر بكثير ($p < 0.05$) مما كانت عليه في ربعي المحايد. كان مستوى تعبير mRNA للعلامة العظمية (Runx2) أكبر بكثير ($p < 0.05$) في الربع المحقون بالكلوروكوين منه في ربع المحايد. تشير نتائج هذا البحث إلى أن الكلوروكوين يمكن أن يعزز إعادة تشكيل العظم ويقلل من الانتكاس بعد تقويم الأسنان في الأغنام.

الكلمات المفتاحية: تقويم الاسنان ، الكلوروكوين ، اسنان الاغنام.