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Clinical and histological evaluation of the effect of magnesium oxide administration on relapse after orthodontic teeth movement (Rabbit Model Study)

Roaa E. Mohammed, Sarmad S. Salih Al Qassar[1] and Ghada A. Taqa

Abstract

OBJECTIVE: This study aimed to evaluate the clinical and histological administration of magnesium oxide (MgO) supplementation on orthodontic relapse and bone remodeling.

MATERIALS AND METHODS: Twenty male albino rabbits were classified into four groups (five animals for each as two control (positive and negative), plus two experimental (low dose 40 mg/kg) and (high dose 80 mg/kg)/b.w. daily). An orthodontic force was applied (40 gm) to the lower incisors using modified orthodontic appliance adapted on the lower central incisors. During the period of retention, MgO was given orally. Relapse was estimated after appliance removal. A digital Calliper was used to compete the space between incisors' mesial tips of rabbits at six successive time points (0, 3, 7, 10, 15, and 21 days). Histologically, osteoblast, osteoclast, and osteocyte account were assessed. Data analyses were performed by SPSS using ANOVA and Tukay HSD ($P \leq 0.05$) for statistically significant differences between groups.

RESULTS: The high dose group had a lower relapse rate than the low dose and control groups. Histologically, the high dose group had more osteoblasts and osteocytes than low dose and control groups. While osteoclasts were significantly lower than the control group in low and high dose groups.

CONCLUSIONS: MgO supplementation during an orthodontic retention phase, particularly at a level of high dose, clinically decreased orthodontic relapse in a rabbit model. Histologically, MgO has a significant effect on alveolar bone after the orthodontic retention period.

Keywords:

MgO, orthodontic teeth movement, osteoblast, osteoclast, relapse

Basic Science Department, [1]Pedodontics, Orthodontics and Preventive Department (POP), College of Dentistry, University of Mosul, Mosul, Iraq

Address for correspondence:
Dr. Sarmad S. Salih Al Qassar,
Collage of Dentistry,
University of Mosul, Mosul, Iraq.
E-mail: sarmadsobhi@uomosul.edu.iq

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Introduction

Orthodontic appliances (OA) are widely used for the correction of malpositioned teeth and to improve dental occlusion and facial esthetics.[1] The mechanism of these corrections depends on the application of light force over the teeth to their suitable position in the dental arch. When such a force is exerted, the teeth push through the periodontal ligament (PDL) and apply compression to the surrounding bone in the direction of the force applied. In contrast, the PDL and the bone in the opposing site of the applied force endure tension. Such changes in mechanical loading are linked to biological changes, which are manifested as cellular changes in response to the new situation. Such cellular changes of the bone around the moving teeth are known as bone remodeling.[2] This remodeling process includes both bone demineralization and remineralization.[2] The coordination of the many phases of this process (activation,

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resorption, reversal, creation, and termination) is known as bone metabolism.[3]

After the orthodontic treatment (OT) is completed, the stabilization of the teeth in their target position is essential.[3] Orthodontic relapse is one of the challenges in the post-relapse phase, and many attempts have been made to face these challenges either by equalization of chewing force distribution[4,5] or by improvement of bone density around the moved teeth using pharmacological products or natural substances. These products are made from pharmacological components that come from natural products or are made by mixing chemicals in a lab.[6]

Magnesium is the fourth most common mineral in the human body, after calcium, sodium, and potassium, and the second most common intracellular cation after potassium. Magnesium is an essential element in a wide range of biochemical reactions, from energy generation to nucleic acid synthesis.[7] Magnesium is required for the conversion of vitamin D into an active form for calcium absorption, and it also stimulates the calcitonin hormone, which transports calcium from the blood and soft tissues back to the bone, protecting bone structure and enhancing bone metabolism.[8] In contrast, it was reported that a deficiency in magnesium has been connected with impaired bone formation, osteoporosis, bone fragility, vitamin D deficiency, and parathyroid hormone deficiency. Magnesium deficiency in men has been linked to accelerated bone loss or decreased bone mineral density (BMD).[9]

According to our data, no previous effort has been completed to investigate the impact of Mg^{+2} supplement administration during orthodontic intervention, mainly its role in improving bone metabolism, in post OTs i.e., relapse phase. Thus, the main goals of this research were to assess clinically the role of different recommended dosages of magnesium oxide (MgO) administration on teeth relapse following an active orthodontic relapse period beside histological assessments of these doses on bony cell counts (osteoblast, osteocyte, and osteoclast) around the moved teeth using OA.

Materials and Methods

To conduct the above aims, rabbits were selected as a living model for this investigation. White male albino rabbits that lived in the animal household of the Dental school of the Dentistry\ University of Mosul were recruited for this experiment; where they existed for at least two weeks, before starting the experiment. The research ethical committee of the University Mosul\ Dental School has read and approved the research protocol (Approval No. Mosul. Dent\ L. A. 55\22). The following formula was performed to calculate the sample size:

[n = (z r/D) 2]. (95% confidence)

Where: n = sample size required; z (constant) = 1.96 units; r = 0.16.[10] D (precision) = 0.2 units. After adjusting the resulted number, the final sample size was computed as the final sample size per group. According to the above, each group had a determined sample size of five animals.

The datasheet for each animal was prepared according to the protocol of this study, which included (number, group, weight, anesthetic dosage, MgO dose, date of OA placement, orthodontic Retention start date, OA de-bonding date, and sacrifice date). As soon as the sample size was determined, twenty white male albino rabbits (age range: 6–8 months; weight range: 1100–1450 gm) were separated into the specified four metal cages according to the study design. They were maintained on a 12:12 light/dark schedule and received 225 g of pellets (Albers® Rabbit 16% Animal Feed) per day. Each rabbit's weight was monitored each week throughout the study. To rule out the influence of food type on the results, the entire sample was given a same diet. All rabbits were fed leafy greens and a regular concentrated pellet diet that were measured per kg per day during the whole study. The water is available at any time. The animals were divided by a simple random method and divided into four groups of five animals each.

Study design

According to the aims of this study, two doses of MgO were tested (High and Low). Thus, total study groups were classified as follows:

Control groups

The control group was subdivided into two groups.

C^{-ve}: where, no magnesium supplementation, where intake for four weeks, no OA, and distilled water were given in the same manner of magnesium taken to the other groups.

C^{+ve} group: control positive group with OA given distilled water only without magnesium supplementation. The relapse was estimated at (0, 3, 7, 10, 15 and 21 days) after the OA de-bonding.

Treatment groups

The treatment group was subdivided into two (2) groups; where these two groups had OAs with MgO supplementation as follows:

LD group: Low-dose group of magnesium 40 mg/kg orally administered one week before appliance debonding until last day of experiment.[11]

HD group: received a high dose of magnesium at 80 mg/kg orally administered one week before appliance debonding until last day of experiment.[12]

Thus, the total sample size was 20 rabbits, as 5 per each group according to the sample size equation see above.

Orthodontic appliances

The rabbits' lower central incisors were fitted with a set of modified fixed OA.

For one week, each rabbit received orthodontic teeth movement, followed by a one-week retention period. Each appliance consisted of two lower central incisor bands (Dentaurum, Ispringen, Germany), size 000, a sectional arch wire (the 0.017 x 0.025 stainless steel wire, and a continuous type nickel titanium open coiled spring (International Orthodontic Services (IOS), USA), which was activated to attain 4.5 mm space and inserted between the two lower incisors bands to generate a force on the two adjust teeth (40 gm). The brackets were attached as close to the gingiva as possible to reduce traumatic occlusion.

Before the spring was adapted, a tension gauge was used to figure out the force in it. When the springs were used, they applied pressure on the distal sides of the treated incisor roots with a force equal to 40 ± 2 g. This applied pressure on the distal surfaces of the treated roots and generated tension in the PDL of its mesial surface. Before the appliance was inserted, ligature wire (Dentaurum, Ispringen, Germany) was used to hold each wire in place in the bracket slots. A mixture of xylazine 5 mg/kg IM and ketamine 35 mg/kg IM was injected into the animal›s muscles to make them relax. Then, a glass-ionomer cement (Tokuso Inomer, Tokoyama, Japan) was used to cement bands of the animal's lower incisors. At the start of the experiment, the OA was already activated, and it didn't get activated again during the experiment.

One weeks after the end of OT, a space was gained between the two lower incisors. The open coil spring of the OA that act as a retainer for one weeks was coated with cold-cured acrylic resin (Major, Italy), which permit rabbits' bones repair. At the end of the orthodontic R period, the band-removal pliers were used to remove the OA. For each group, orthodontic relapse was assessed with a digital caliper at 3, 7, 10, 15, and 21 days.

Magnesium supplementation

The magnesium supplement in the form of MgO tablet (250 mg tablet, 21st century health care, AZ 85282 USA) was forcefully administered to the treatment group orally to ensure complete administration of the prescribing dose by mixing one tablet with 5 ml of distilled water according to body weight for the LD group, each 1 ml containing 50 mg of magnesium. 0.8 ml was administrated for the LD group and 1.6 ml/kg was administrated for the HD group. The MgO was given once daily at 12 p.m. starting one week before appliance de-bonding by the scarifying date.

Assessment of body weight

All animals were weighted at day 0, 1st, 3ed week, 5rd week, 6th and 7th week of the experiment. The dose of MgO was designed according to the body weight and to evaluate the effect of the MgO on the body weight.

Assessment of relapse

A digital caliper (Pro-Max, China) was used to quantify the amount of relapse distance between the mesial points of the lower incisors, with a minimum detectable distance of 0.01 mm. The measurements days were taken as: on days 0, 3, 7, 10, 15 and 21. The relapse distance rate (RD) was measured as the following formula (RD = sp0-sp1). Where, the space 0 (sp0) at the time of de-bonding, while, the sp1 represent the space on the day of measurement (0, 3, 7, 10, 15 and 21). All the measurements were repeated by two experienced researchers (first and second authors) who were oblivious to the applied regimen and had to repeat it three times. In their analysis, the examiners had a high level of agreement (= 0.81), indicating that intra-examiner and inter-examiner reliability was satisfactory. As a representative value for each distance, the mean of these measurements was chosen.

Scarifying the rabbits and preparing of the histological assessments

Scarification was performed in a sterile condition on the operating table after total anesthesia had been obtained. The rabbit was placed on its back, and the hair above the lower jaw in the surgery area was removed with a manual hair clipper. As an antiseptic, the shaved portion is rinsed with povidone iodine and wrapped in a sterile towel around the surgical area. An incision of about 1.5 cm was made over the mandibular bone with surgical blade no. 15. Using the Hawarth periosteal elevator, the periosteum was lifted and exposed to clean bone. Dissection of the bone around the target teeth was done from the pressure side, and it was inserted in 10% neutral buffered formalin for 48 hours to prevent its break down. Subsequently, Decalcified and dehydrated specimens were imbedded in paraffin wax and coated. Longitudinal serial sections were performed for each specimen at a thickness of 5 mm. For each specimen, dissection was carried out in a mesio-distal direction, parallel to the root of the lower incisor's long axis. On the pressure side, the specimens were stained with H and E.[13] Each tooth's long axis was dissected longitudinally into 5-m thick histological sections.

Histological assessment

Quantitative valuation of histological sections

Alveolar bone at the coronal, middle, and apical third of the root pressure side of bi lateral lower central incisors was examined at a power field (objective lens) of 400x for precise assessment of each histological slice, and cells were calculated and expressed as cell counts per microscopically power.

Counting the number of osteoblasts

On the surfaces of actively growing bone, a plump cell with basophilic cytoplasm and an eccentric nucleus was identified as an osteoblast.[14] The number of osteoblasts visible in the field of view at a microscopic magnification (objective lens 400x).

Counting the number of osteocytes

Osteocytes were observed residing in a tiny area inside the bone called a lacauna, which had canaliculi coming out of it.[14] At a microscopic power (objective lens 400x), osteoocytes were visible in the field of view.

Counting the number of osteoclasts

The osteoclast seemed to be a big multinucleated cell with a curved surface. At a microscopic power (objective lens 400x), it requires a microscope with a high-power lens to observe the osteoclasts in the field of view.[14]

Micro-morphometric measurement

The color USB 2.0 digital image camera (Omax ToupView 9.0-Megapexil China), which included with image processing software, was used to test all variables. All of the Microscope-Olympus-lenses CX31's were calibrated to the camera's software using a 0.01 mm stage micrometer (ESM-11/Japan).

Statistical analysis

The descriptive analysis (Mean and Stranded deviation) of the data for the measurement of weight, relapse distance, and bone cell count among different groups was performed. Additionally, A Shapiro-Wilk test was performed to evaluate the normality of the data. For detection of the significant differences between the data, ANOVA and Tukay HSD statistical tests were performed. All the above tests were done by IBM SPSS Statistics, Version 25 (IBM Corporation, USA). *P* value was adjusted to be 0.05. Also, ten randomly chosen slides for histo-morphometric analysis was used to detect an intra-class correlation and the reliability of the measurement.

Result

Measurement of weight

A Shapiro-Wilk test (at $P \leq 0.05$) was used to assess the normality of the distribution of the data. In terms of animal status, the Shapiro – Wilk test value was 0.78 which represent normal distribution of the data. No weight loss was observed statistically in any of the groups throughout the study, and there were no significant differences in weight gain between groups with high dose (1.45 ± 0.02) kg, low dose (1.1 ± 0.083) kg, and control positive (1.35 ± 0.02) kg. $P = 0.099$ as shown in Table 1.

Clinical findings

The degree of orthodontic relapse for each group experienced was determined by the dose that were given. The Shapiro-Wilk test was employed to determine the normality of the data distributions (0.56). Following the removal of the appliances, all of the animals showed signs of moving teeth to their original position, three days after the appliance was removed, and a rapid relapse began. The relapse rate gradually declined until day 21, the last day of the experiment, after de-bonding. A measurement was taken at the level of the lower central incisor, which was the location of the measurement. Table 2 shows how different the groups were in terms of how often they relapsed. After de-bonding, the HD group had a considerably lower relapse rate (P 0.05) than the other two groups. Table 2 also demonstrates a trend toward greater relapse distance, which was lower in the HD and LD groups than in the control group. On day 21, the HD group significantly reduced post-orthodontic relapse ($P \leq 0.05$) from mm overall mobility in the control group to mm in the LD group. The difference between the high and low doses was considerable. Relapse is measured in a variety of ways, as illustrated in the Table 2.

Statistical analysis of the osteoblasts counts

The statistical analysis revealed that there was a significant variation in the three levels of the root within each group (Coronal, Middle and Apical). In the coronal part of the bone, there was a significant increase in the number of osteoblasts found in both the high dose (30 ± 2.1) and low dose (29 ± 1.7) groups in comparison with the control positive group (10 ± 0.6) decrease, while, The control group had the normal number, as shown in Table 3. Also, there was a significant increase in the number of osteoblasts found in the middle part of the bone by both the high dose (32 ± 2.4) and low dose (30 ± 3.2) groups when compared to the control positive (9 ± 0.9) and control negative (28 ± 1.3) groups, which was normal, as shown in Table 3. In the apical part of the bone, there was a significant increase in the number of osteoblasts founded in both the high dose (53 ± 3.9) and low dose (41 ± 2.7) groups in comparison with the control positive group (15 ± 0.8) and the control negative group (40 ± 3.5), which was normal, as shown in Table 3.

Statistical analysis of the osteoclasts counts

In the coronal part of the bone, there was a significant increase in the number of osteoclasts found with the

Table 1: Descriptive analysis (Mean and standard deviation) and ANOVA-test comparisons of rabbits weight measurements in kg throughout the study

Group	Mean±SD						*P*
	Day 0	Week 1	Week 3	Week 5	Week 6	Week 7	
C +ve	1.47±0.02	1.3±0.01	1.35±0.015	1.3±0.01	1.47±0.02	1.44±0.021	0.099*
LD	1.33±0.01	1.35±0.076	1.3±0.135	1.18±0.028	1.1±0.083	1.32±0.07	
HD	1.47±0.01	1.3±0.02	1.35±0.01	1.1±0.03	1.4±0.02	1.46±0.15	

*P<0.05, significant differences between groups, Values are presented as mean±standard deviation

Table 2: Descriptive analysis (Mean and standard deviation) of the relapse rate (mm) and ANOVA-test and *post hoc* Tukey HSD statistical results after de-bonding among three tested groups

Group	Day	Mean±SD	*Post hoc*	*P*	Day	Mean±SD	*Post hoc*	*P*
C +VE	0	0	-	-	10	0.2±0.01	A	0.00*
LD		0	-			0.18±0.041	B	
HD		0	-			0.1±0.01	C	
C +VE	3	0.05±0.01	A	0.00*	15	0.24±0.031	A	0.00*
LD		0.07±0.021	B			0.23±0.011	B	
HD		0.03±0.023	C			0.12±0.01	C	
C +VE	7	0.11±0.01	A	0.00*	21	0.3±0.021	A	0.00*
LD		0.14±0.041	B			0.28±0.023	B	
HD		0.07±0.033	C			0.14±0.002	C	

Values are presented as mean±standard deviation. *P≤0.05, Significant differences between groups, Tested by one-way analysis of variance, and *post hoc* Tukey HSD analysis test

Table 3: Descriptive statistics and ANOVA-test and *post hoc* Tukey HSD analysis test of the number of osteoblasts, Osteoclasts and osteocytes found in the trabecular bone in the pressure site (40× field)

Group	Mean±SD				P
	C -ve	C +ve	HD	LD	
Osteoblast					
Corounal	23±2.4A	10±0.6B	30±2.1C	29±1.7C	0.00*
Middle	28±1.3A	9±0.9B	32±2.4C	30±3.2A	0.00*
Apical	40±3.5A	15±0.8B	53±3.9C	41±2.7A	0.00*
Osteoclast					
Corounal	2±0.05A	5±0.3B	2±0.08A	2±0.09A	0.039*
Middle	2±0.04A	5±0.1B	2±0.06A	1±0.03A	0.024*
Apical	3±0.09A	6±0.7B	4±0.04A	2±0.05A	0.031*
Osteocyte					
Corounal	45±4.1A	26±2.6B	35±2.5C	27±1.3B	0.00*
Middle	40±3.3A	22±1.1B	44±2.7A	39±3.1A	0.00*
Apical	35±2.8A	23±1.9B	32±2.5C	34±2.2C	0.00*

Values are presented as mean±standard deviation. *P<0.05, Significant differences between groups, Tested by one-way analysis of variance, and *post hoc* Tukey HSD analysis test, different letters represent significant differences between groups

control positive group (50 ±0.3), compared to a decrease in both the high dose (20 ±0.08) and low dose (20 ±.09), with the control negative group (20 ±.05) being normal, as shown in Table 3. In the middle part of the bone. The number of osteoclasts formed in the control positive group (5 ± 0.1) was significantly higher than in the high dose (2 ± 0.06) and low dose (1 ± 0.03) groups, while the control negative group (2 ± 0.04) was normal. There was a significant increase in the number of osteoclasts formed in the apical part of the bone with the control positive group (6 ± 0.7), compared to a decrease in the number of both high dose (4 ± 0.04) and low dose (2 ± 0.04), control negative group (3 ± 0.09), which, was normal, as shown in Table 3.

Statistical analysis of osteocytes count

The statistical analysis revealed that in the three levels of the root, there was a significant difference between each group (Coronal, Middle and Apical). There was a significant increase in the number of osteocytes found in the high dose (35 ± 2.5) compared to the decrease in number in both the low dose group (27 ± 1.3) and the control positive group (26 ± 2.6), while the control negative group (45 ± 4.1) was normal. There was a significant increase in the number of osteocytes found in the middle part of the bone in both the high dose (44 ± 2.7) and low dose (39 ± 3.1) groups when compared to the control positive group (22 ± 1.1) and the control negative group (40 ± 3.3), which is normal. There was a significant increase in the number of osteocytes founded by both the high dose (32 ± 2.5) and low dose (34 ± 2.2) groups in comparison to the control positive (23 ± 1.9) and control negative (35 ± 2.8) groups, which are normal, as illustrated in Table 3.

Histological finding

Sections of the bone around the tooth in the control negative group showed the coronal side with normal architecture of PDL with fibroblast (PL) and trabecular bone (TB) surrounding the tooth, as well as the trabecular bone surrounding the tooth with normal architecture of osteoblast (OB), osteocytes (OC), and osteoclast (OCL), while the middle side showed the normal architecture of PL with fibroblast and TB. Normal architecture of OB, OC, and OCL on the apical side with normal architecture

of PDL with fibroblast (PL) and TB surrounding the tooth, also trabecular bone surrounding normal architecture of OB, OC, and OCL.

In the control positive group, a section of bone surrounding the tooth showed the coronal side represented by thickening of PDL, congestion blood vessels (C), and mild inflammatory cell infiltration surrounding it (I) of trabecular bone surrounding the tooth showed normal architecture of OB, OC, and OCL; the middle side represented by thickening of PDL with hemorrhage, erosion in the edge of TB congestion blood vessels, and mild inflammatory cell infiltration surrounding it, as shown in Figure 1.

The coronal side of the tooth was represented by congestion of blood vessels in the PDL, increased numbers of OB in the trabecular bone, and growth of newly woven bone formation (WB) in the low dose group. The middle side is represented by the filling of an eroded part of trabecular bone with granulation tissue containing immature connective tissue and angiogenesis. Trabecular bone surrounding tooth shows normal architecture of OB, OC, and OCL. Apical side represents the presence of congestion of blood vessels in the PDL and the presence of newly WB formation surrounding TB, which shows normal architecture of (OB), (OC), and OCL as shown in Figure 2.

In the high dose group, the coronal side of the tooth was represented by congestion blood vessels in the PL and increased numbers of OS in the TB surrounding the tooth, while the middle side was represented by increased numbers of blood vessels in the PL and increased numbers of OC in the trabecular bone OB. bone surrounding the tooth shows normal architecture of OB, OC, and OCL and the apical side represents the presence of granulation tissue (GT) with immature connective tissue (ICT) and angiogenesis (An) and newly woven bone formation (WB). Trabecular bone surrounding tooth show normal architecture of OB, OC and OCL, as shown in Figure 3.

Discussion

Retainers are utilized by clinicians after active orthodontic intervention to prevent relapse and preserve teeth in their new position. However, long-term retainer use requires patient compliance. Therefore, it is vital to create techniques that could enhance the stability of teeth throughout the retention phase.[13] The MgO supplementation was used in this study, as it is a simple way for both the clinicians and patients in order to improve the bone metabolism around the moved teeth. On the pressure side, histological reactions in AB tissue components were examined to evaluate the value of MgO

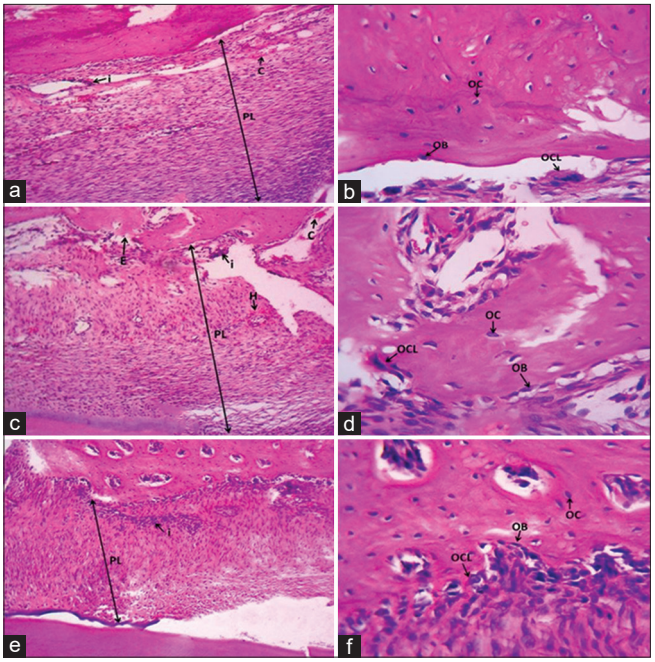

Figure 1: Illustrated Microscopic examination of tissue sections by, H and E stain, 400X revealed presence of (a) the coronal side by thickening of periodontal ligament (PDL), congestion blood vessels (C) and inflammatory cells infiltration. (b) coronal side normal architecture of osteoblast (OB), osteocytes (OC) and osteoclast (OCL), H and E stain, 400X (C) middle side by thickening of periodontal ligament (PL) with hemorrhage (H), erosion in the edge of trabecular bone (E), congestion blood vessels (C) and mild inflammatory cells infiltration surrounding it. (d) middle side with normal architecture of osteoblast (OB), osteocytes (OC) and osteoclast (OCL) (e) apical side with thickening of periodontal ligament (PL) and severe inflammatory cells infiltration surrounding it, inflammatory cells infiltration surrounding it, (f) apical normal architecture of osteoblast (OB), osteocytes (OC) and osteoclast (OCL)

administration on the osteogenicity of the alveolar bone, where, bone de-mineralization and remineralization processes were performed.

Animal studies can support learn about the effects of natural products and drugs on the bones as well as to avoid the complications that could occur in human applications. All the rabbit groups used in this study lived in the same conditions. Rabbits are cheap and have great bone turnover, as their bone remodeling period is completed in only six weeks. While, in humans it takes about 3–6 months.[15] Since, they were the most commonly used species in bone studies.[16] The goal of the select seven days of orthodontic tooth movement was to promote rapid bone turnover. In addition, previous studies[17,18] have observed histological changes that occur when animal undergoes OT for seven days. This study was performed on the lower incisors because they are easier to reach as they are in the front of the mouth. In this way, orthodontic relapse can be easily observed and measured. Additionally, OA for lower incisors were less distressing for the animals than other appliances were if they were constructed posteriorly between the first molars and the lower incisors.[17] The males' rabbit was recruited in this study in order to exclude hormonal

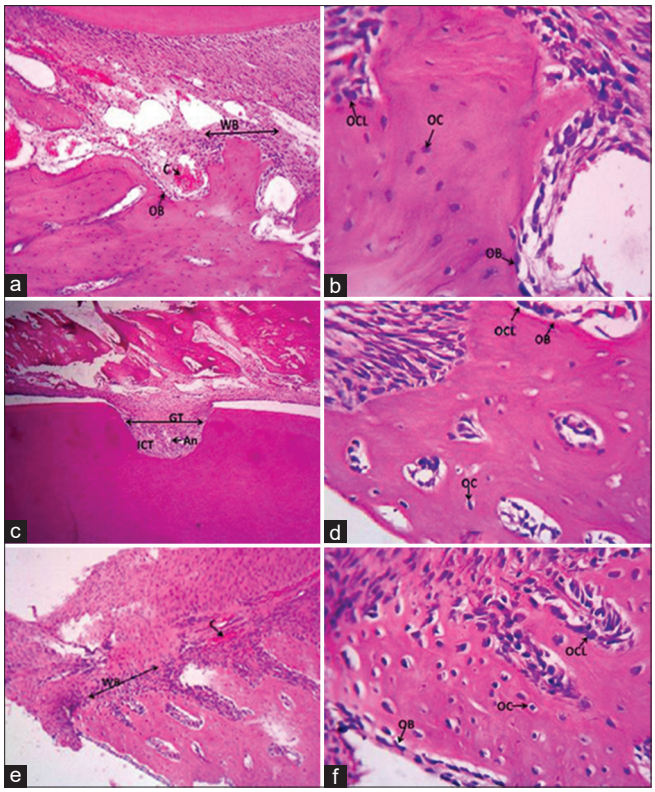

Figure 2: Illustrated Microscopic examination of tissue sections by, H and E stain, 400X revealed presence of (a) coronal side representing by congestion of blood vessels in the periodontal ligament (C), increased numbers of osteoblasts in the trabecular bone (OB) and growing of newly woven bone formation (WB), (b) coronal side with normal architecture of osteoblast (OB), osteocytes (OC) and osteoclast (OCL), (c) middle side with filling of eroded part of trabecular bone with granulation tissue (GT) containing immature connective tissue (ICT) and angiogenesis, H and E stain, 400X inflammatory cells infiltration surrounding it, (d) middle side with normal architecture of osteoblast (OB), osteocytes (OC) and osteoclast (OCL) (e) apical side with congestion of blood vessels in the periodontal ligament (C) and presence of newly woven bone formation surrounding trabecular bone (WB), inflammatory cells infiltration surrounding it, H and E (f) apical side with normal architecture of osteoblast (OB), osteocytes (OC) and osteoclast (OCL), H and E stain, 400X

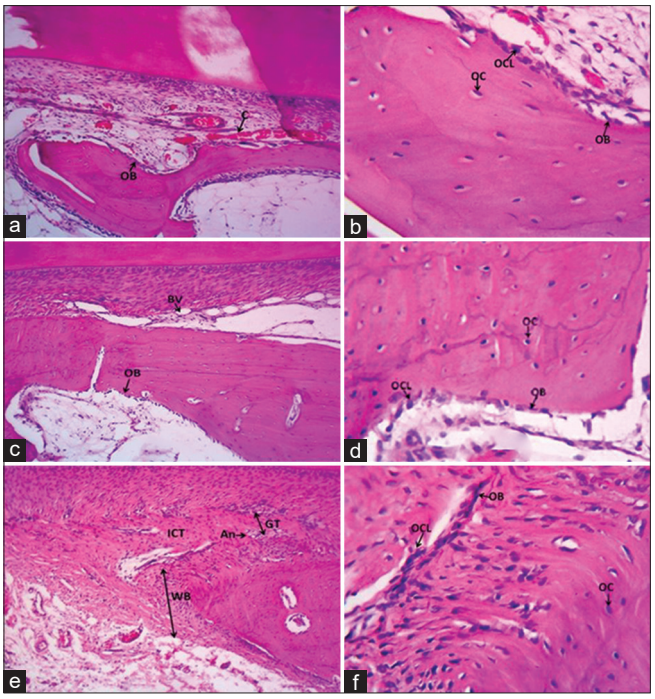

Figure 3: Illustrated Microscopic examination of tissue sections by, H and E stain, 400X revealed presence of (a) coronal side with congestion blood vessels in the periodontal ligament (C) and increased numbers of osteoblasts in the trabecular bone (OB), (b) coronal side with normal architecture of osteoblast (OB), osteocytes (OC) and osteoclast (OCL) (c) middle side with increase numbers of blood vessels in the periodontal ligament (BV) and increased numbers of osteoblasts in the trabecular bone (OB), (d) middle side with normal architecture of osteoblast (OB), osteocytes (OC) and osteoclast (OCL), (e) apical side representing by present of granulation tissue (GT) with immature connective tissue (ICT) and angiogenesis (An) and presence of newly woven bone formation (WB), (f) apical side with normal architecture of osteoblast (OB), osteocytes (OC) and osteoclast (OCL)

changes that are usually observed in females. Rabbits' nutrition was the same all through the study with two weeks of accommodation period before starting the experiment. Also, all rabbits got the same daily feed. No significant variation in their weight was observed in spite of the diarrhea side effects of Mg^{+2} that were previously reported.[18] The rabbits were forcefully administered MgO supplement in order to ensure full dose administration by the rabbits. However, this procedure may reflect some anxiety behavior in the animals. Nevertheless, MgO is used as an anti-stress drug.[19] The emphasis of our histology investigation was on the pressure side. The tension side of OTM becomes the pressure side during relapse, resulting in bone resorption. Former pressure is transformed into tension, which promotes osteoblast differentiation and new bone development.[10]

According to the results of the study, relapse was observed in all groups following appliance de-bonding. Immediately after de-bonding, all groups showed a higher relapse rate, which gradually reduced in the coming 21 days. This conclusion compared with the findings of Franzen *et al*.,[20] who found that after the appliance was removed, the relapse of moved teeth began quickly. Orthodontic relapse appears to occur rapidly, particularly during the first week following appliance removal. Bone resorption persists on the previous pressure side (mesial side) during the early stages of orthodontic relapse, but bone synthesis appears to begin only a few days following the release of orthodontic tension. This phenomenon may be explained by blood vessel cells apoptosis and reduced angiogenesis that happen within a few days after bracket de-bonding.[20] At the 21st day after de-bonding, there is only a slight relapse tendency; the teeth have regained their original physiological position after stabilizing.[10]

In the current study, a significant difference was found between HD and LD of treatment groups in comparison to the control group in relapse rate, this could be explained by the fact that the treatments groups have a reduced relapse rate. In all three-thirds of the dissected region, the treatment group had considerably

greater osteoblast counts, indicating the initiation of positive bone remodeling. Mg^{+2} has been linked to the mineralization process, particularly during the early stages of osteogenesis, and hence has an impact on the mechanical behavior of bone. Mg^{+2} is also necessary for cellular and enzymatic functions, and it increases cell adhesion, proliferation, and metabolic activity. Also, Mg^{+2} is one of the most common calcium substitutes in biological apatite.[21] Increased Mg^{+2} dose (80 mg/kg b.w. daily) had a favorable effect on alveolar bone in the post-orthodontic relapse period, as evidenced by osteoblasts and osteocytes in all three thirds. The fact that the treatment group had more osteoblasts than the control group shows more than just better osteogenic differentiation. As well as suggesting advanced stages of bone remodeling. The histological findings for the control group versus the HD and LD groups in this study revealed a significantly lower number of osteoclasts at all third parts, representing the beneficial roles of increasing Mg^{+2} dose (80 mg/kg b.w. every day) In the post-orthodontic relapse stage, on alveolar bone The treated group's decreased osteoblast number comparison to the control group shows enhanced osteogenic differentiation as well as reduced bone resorption. Other explanations for the effects of Mg^{+2} on the bone demonstrate a possible Mg^{+2} stimulation of the intracellular signaling pathway in hBMSCs that may lead to the enhanced ECM mineralization that was observed *in vitro* and the enhanced osteogenesis that was observed in vivo.[22] However, numerous studies have evaluated the osteogenic activity of Mg^{+2}, but few have explored the impact of these ions at varied doses.

Osteoblast outcomes

Regarding osteoclast account in the coronal part, there is no significant between LD and HD. While In the middle part, there are a higher number of osteoblasts in the HD group than in the LD group. In the apical part, there is a significant difference between HD and LD.

This research confirmed previous work that Mg^{+2} has favorable effects on osteoblast development, proliferation, survival and function.[7,23] This could be contributed to the important function of magnesium in bone remodeling, which is linked to the adherence and proliferation of osteoblastic cells, stimulating expression of growth factors and early osteogenic markers that induce bone formation.[24] These results are in agreement with Orchard *et al*.,[9] Al Alawi *et al*.,[25] as they demonstrate an increased bone regeneration by Mg^{+2} supplementation. Magnesium plays an important function for the development of osteoblastic cells and positively affects BMD. It was reported that Mg^{+2} ion is mitogenic for osteoblasts.[26] Thus, increased Mg^{+2} availability in turn stimulates cell growth.[26] In this study, higher concentrations of Mg^{+2} demonstrated higher impacts on the viability of osteoblasts. Our results are in agreement with He *et al*.,[27] as increase in intracellular magnesium might enhance the proliferation and migration of human osteoblast MG-63 cells.[27]

During bone metabolism, Mg^{+2} ions attach to the surface of hydroxyapatite crystals, increasing the solubility of Pi and Ca^2 hydroxyapatite and, as a result, influencing crystal size and formation. Mg^{+2} also promotes the growth of osteoblasts.[27] Mg^{+2} deficiency, on the other hand, causes decreased bone creation and osteoblast counts, as well as lower bone mass, Mg^{+2} effects include osteoblast activity and count, as well as hydroxyapatite crystal formation and, finally, calcium homeostasis regulation. Previous studies[28] also demonstrate that the Mg^{+2} transporter Magt1 that plays an important role in the odontogenic differentiation of bone marrow MSCs by regulating intracellular Mg^{+2}.

Osteoclast outcomes

According to our results, there is no significant difference between the LD and HD number of osteoclasts in all three thirds of the root. The impact of Mg^{+2} in bone healing can be attributed to the release of Mg^{+2} ions that affect both osteoclast and osteoblast function for enhanced bone remodeling. In this manner, stimulate the release of transforming growth factor-1 (TGF-1) which can induce bone marrow stem cell (BMSC) migration for neo-tissue growth.[29] Furthermore, the stimulation of Mg^{+2} ions may increase the quantity of osteoclast precursors (osteocytes).[29] This match with the Belluci *et al*. results[30] who work on rat and mice models, as they found that Mg^{+2} deficient suggests increased bone resorption as a cause in the decrease of bone mass.[30]

From another hand, the amount of osteoclasts created from bone marrow precursors is inversely connected to Mg^{+2} deficit, according to research: the lower the extracellular Mg^{+2} quantity, the more osteoclasts are formed.[31] It was discovered that a lack of Mg^{+2} ions in the bone was associated with either decreased or diminished osteoclast activity.[32] It is important to note that a larger number of osteoclasts does not result in an increase in their resorptive activity. In fact, less activity was seen per osteoclast. The increased number of osteoclasts could be an attempt by the cells to compensate for their decreased ability to resorb calcium. This compare with certain hereditary models of osteopetrosis, which show a higher number of functional osteoclasts but a lower number of functional osteoclasts. Thus, others showed that a lower Mg^{+2} level increases the production of osteoclasts with limited activity. The clear mechanisms behind Mg^{+2} action on osteoclasts are unknown yet.[33] In human osteoclasts, cell proliferation and differentiation were elevated at low Mg^{+2} concentrations and decreased at relatively high Mg^{+2} concentrations. They also

determined the significance of the Mg^{+2} origin, As a result, Mg^{+2} reduction may impair cellular metabolism and an increasing proportion of osteoclasts might be owing to the cells' effort to compensate for their decreased capacity to resorb.[34] Rude *et al*.[35] have shown that Mg^{+2} deficiency increases the number of osteoclasts in rats. In vivo, magnesium insufficiency affects not just bone metabolism but also the immunological and other past *in vitro* results indicate a direct influence on osteoclast production.[35,36]

On the other hand, Mg^{+2} depletion was found to upsurge the secretion of pro inflammatory cytokines such tumor necrosis factor (TNF), interleukin (IL)-1, and substance P, which have all been linked to increased osteoclastic bone resorption. Reduced parathyroid hormone (PTH) and 1,25 (OH) 2D3 levels, which are commonly linked with hypomagnesemia, may exacerbate these effects.[36] Mg^{+2} deficiency enhances the inflammatory response by increasing the synthesis of cytokines and growth factors involved in the formation and activation of multinucleated osteoclasts, the body's bone-breaking cell.[36] Cytokines such macrophage colony stimulating factor (M-CSF) and the osteoclast differentiation factor RANKL, as well as their ligands, c-fms and RANK, are involved in the development and function of these cells.[36]

Osteocyte outcomes

In the coronal part, there is a significant difference between LD and HD. In the middle and apical parts, while there is no significant difference in osteocyte in regard to HD and LD.

Osteocytes are assumed to have a variety of functions, including osteolysis, mineralization control, and mineral metabolism. They can also play a role as mechano-sensory cells, detecting mechanical stimulation produced by mechanical load capacity of bones, and they are responsible for the regulation of osteoblast and osteoclast function during bone remodeling.[37]

During orthodontic tooth movement, the process of alveolar bone remodeling is essential. Osteocytes are hypothesized to control the process of bone remodeling.[38] As a consequence, osteocytes may exhibit a variety of transduction mechanisms and intracellular signaling pathways, such as cytoskeletal alterations or intracellular calcium influx that are related in some way in osteocyte mechano-transduction.[37]

It's possible that human primary osteoblasts' ability to differentiate into osteocytes is donor-dependent. A thorough awareness of bone biomechanics necessitates the recognition of bone quality. Bone quality, unlike other biomechanical measures like bone density and volume, considers the structure and composition of bone, both of which are linked to bone mineral depositing. In vitro, high doses of magnesium accelerated hBMSCs' early osteogenic differentiation but blocked MSC-mediated late calcification. Experiments *in vivo* showed that high magnesium levels interfere with calcification processes such mineral crystallization and mineral-matrix balance, reducing bone quality and biomechanical performance.[39]

The demonstration of magnesium's action on osteocytes provides a new mechanism underlying its beneficial function in promoting bone health.[40]

The impact mechanism of action of Magnesium on bone

Several studies have shown that magnesium is a major component of bone, accounting for 67 percent of the body's magnesium. It improves bone remodeling and suggests that the potentially biodegradable metal magnesium increases bone density.[7,20] It plays an important role in bone metabolism, acting as a mitogen for osteoblasts, which multiply in its presence, as well as a protective factor against excessive bone resorption and a function in bone repair.[40]

Although magnesium has direct physiological effects, such as reducing the circulation of low-grade inflammatory cytokines, it has also been discovered that it plays critical roles in bone formation, growth, and regeneration, and that it is an essential cofactor for enzymatic reactions required for the synthesis of bone matrix constituents.[9]

Magnesium has an indirect effect on mineral metabolism by enhancing alkaline phosphatase activity, and it has a direct effect on mineral production and crystallization processes.[40]

Magnesium facilitates the activation of vitamin D dependent on magnesium bioavailability and is required for the conversion of vitamin D to its active form, which increases calcium absorption and metabolism, as well as proper parathyroid hormone action and improved magnesium absorption through the intestine, forming a feed-forward loop which maintains magnesium's homeostasis,[7,39] by controlling calcium and phosphate balance, it has an impact on bone development and maintenance. Calcium and phosphate ions influence osteoblast function when they are released into the surrounding tissue. Furthermore, high amounts of extracellular calcium promote DNA synthesis in osteoblastic cells while inhibiting osteoclastic creation.[10] Approximately, fifty percent of the body's magnesium is deposited on the hydroxyapatite surface of bone. Consequently, optimizing Mg intake may be an effective and low-cost preventive measure against osteoporosis

in individuals with documented Mg^{+2} deficiency.[40] Since magnesium acts as a natural calcium antagonist, the molecular basis for inflammatory response could be the result of modulation of intracellular calcium concentration.[40] Mg^{+2} is the second element in bone after Ca and part of the Mg on bone surface (30%) is exchangeable, acting as a dynamic store to maintain intra- and extracellular Mg^{+2} levels.[40]

Clinical implication

The clinical implication of this study is mainly to advise the orthodontist for the prescription of Mg^{+2} supplements during the retention period to enhance bone mineralization after active OT as it is cheap and available with limited side effects.

Study limitations

Specifically, the same appliance was used as a retainer during the relapse phase for short retention period. Because all permanent teeth in rabbits are elodont, this model may have major limitations. This was accomplished by cutting the research time in half, to 7 weeks as total.

The H and E stain, which is considered the most basic histological stain for measuring cell and tissue morphology and dispersion, was used in our research. This method, however, has drawbacks, such as stain dilution and contamination, a drop in haematoxylin dye level, and uncontrolled stain, which can result in a loss of contrast or color balance between the haematoxylin stain and the eosin counterstain. Nonetheless, researchers prepared our slides and interpreted the results under the supervision of a professional pathologist, who is the standard method for pathology diagnosis.

The MgO supplementation was given separately from the rabbit's meal. When compared to other procedures such as oral gavages or injections, it significantly reduces animal handling and stress, which can affect their behavior and metabolism, and saves the operator time.

Excessive Mg^{+2} appears to have bad consequences on the bone while misgivings remain about supplementing the general population.

Conclusion

This study shows the effect of orally administered MgO on decreasing the relapse after retention period by increasing osteoblast, osteocyte, and decreasing osteoclast numbers. In this study, MgO was administered for three-week intervals. In MgO administered animals, the relapse tooth movement was reduced significantly, with a lesser number of osteoclasts, and new woven bone formation in low and high dose in compared with control group.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Conflicts of interest

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