



## Evaluating the Systemic Effect of Magnesium Oxide on Gene Expression of Osteocalcin and Vitamin D Receptors in Rabbits with Orthodontic Teeth Movement



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**BACKGROUND:** The Bone is continuously remodeling in response in response to external and metabolic stimuli. It mimics orthodontic tooth movement by altering bone. Magnesium is a main human mineral That helps in bone development **Aim of the study** : to evaluate the systemic effect of Magnesium oxide on bone formation in rabbit with orthodontic teeth movement. **Material and method** : 20 male albino rabbits weighing (1100-1450) gm. Two control (positive and negative) and two experimental groups each had five animals (low dose and high dose). During orthodontics, experimental animals received 40 mg/kg and 80 mg/kg of MgO daily. Lower central incisors of rabbits received fixed orthodontic devices for three weeks. All study groups sacrificed animals at the end. The lower jaw was isolated and section bone specimens were taken from the site of relocated teeth, gum, and periodontal ligament for quantitative PCR (QPCR). SPSS used ANOVA and Tukey tests to find statistically significant differences **Result** : No significant changes at  $p \leq 0.05$ . Regarding serum Ca<sup>2+</sup>, Mg<sup>2+</sup> and alkaline phosphatase) in treatment group compared to control groups, however genomic study showed that there was an increase in the quantitative gene expression of both osteocalcin and vitamin D receptors at ( bone , gum and periodontal ligament ) in the regarding low dose magnesium -treated group than in the control group and high dose group at the end of study periods. **Conclusion** : magnesium low dose increase bone formation and regeneration at the bone sites of orthodontic teeth movement more than the control untreated rabbits and high dose group.

**Keywords:** Magnesium oxide, Gene expression, Bone formation, Orthodontic appliance.

### Introduction

Bone is the living tissue that constitutes the skeleton of the body; Bone remodeling is a phenomenon that typically happens in response to both environmental stimuli and metabolic changes. Resistance to external forces, wound healing, fractures, and calcium and phosphate homeostasis are all achieved by bone remodeling[1]. Bone provides the perfect balance

of stiffness, flexibility, and lightness since it is a composite material consisting of minerals and collagen. The skeleton is one of the major organs and systems in the human body, making up equal to about 15% of the ideal body weight (although only 30% of the weight of the human body) The process of bone remodeling is biologically governed by systemic and immunological mediators [2].

During orthodontic treatment, a tooth can be forced through the periodontal ligament if adequate orthodontic force is employed. This is based on the premise that a change in mechanical loading of biological systems induces strain, which subsequently drives cellular processes to adapt the system to the new conditions. As a result, when orthodontic pressure is applied to a tooth, the periodontal ligament (PDL) and alveolar bone around it remodels [3]. According to a recent study on recurrence, only 30 to 50 percent of orthodontic patients maintained their post-treatment alignment over a 10-year period. Studies involving pharmacologic therapy and biomaterials have been proposed in an effort to offer another mechanism to improve the stability of teeth following orthodontic treatment [4].

Magnesium is essential to bone metabolism via a variety of processes. Osteoporosis is the result of an imbalance between bone formation and resorption [5]. It is a vital nutrient that affects bone health. This nutrient aids in bone formation by promoting osteoblast proliferation and serving as a vital cofactor for the enzymes required for the creation of bone matrix. Additionally, a lack of magnesium can result in abnormal hydroxyapatite crystals, which are a significant part of bone, as well as an increase in the release of pro-inflammatory cytokines, which stimulate the activity of osteoclasts and lower levels of parathyroid hormone (PTH) and 25-hydroxyvitamin D [25 (OH) D]. It is still unknown whether magnesium can have the same effect on osteoporosis progression as calcium and vitamin D [6]. The bones contain 67 percent of the magnesium in the body, they are an important source of magnesium. In fact, a strong correlation has been discovered between consumption of  $Mg^{+2}$ , an important and variety of micronutrient with a variety of metabolic, structural, and regulatory roles, and bone density [7].

Magnesium, a promising biodegradable metal, It has been shown to promote bone development in a variety of research. Although there is little knowledge of the levels of magnesium ions that influence cellular bone remodeling [8]. It is important for bone metabolism because it acts as a mitogen for osteoblasts, which cause them to multiply when there is enough magnesium, and as a protector against too much bone resorption [9]. It aids in vitamin D activation, which controls calcium and phosphate homeostasis to affect

bone formation and. As a cofactor, magnesium helps enzymes work in the liver and kidneys. All of the enzymes that break down vitamin D seem to need magnesium. The bioavailability of magnesium is crucial for these different steps of vitamin D conversion [10].

The vitamin D receptor (VDR) gene encodes the vitamin D receptor (VDR) protein, which enables the body's response to vitamin D. This vitamin can be obtained from food or manufactured by the body with the aid of sunlight. Vitamin D serves to maintain the right balance of various minerals, including calcium and phosphate, which are important for the normal development of bones and teeth. Controlling the uptake of calcium and phosphate from the gut into the bloodstream is also one of vitamin D's primary functions. Vitamin D is also engaged in processes linked to bone and teeth development [11]. Despite the fact that osteocalcin is created by osteoblasts, it is frequently employed as a marker for the bone production process. During treatment with osteoporosis medications that promote anabolic bone production, such as teriparatide, increased serum osteocalcin levels have been reported to correlate reasonably well with increases in bone mineral density. In numerous studies, osteocalcin serves as a preliminary indicator of a drug's impact on bone growth [12-14].

However, little is understood about how magnesium affects bone formation after orthodontic tooth movement. The purpose of this study was to analyze biochemical parameter (serum alkaline phosphatase, serum calcium and serum magnesium) and to form a genomic study on osteocalcin and vitamin D receptor at (bone, gum and periodontal ligament) in order to explore the effects of magnesium supplementation on bone formation in a rabbit model with modeled orthodontic tooth appliance.

## **Material and Methods**

### *Experimental model*

Rabbits were picked as the living model for this research to achieve the aforementioned goals. Previous to the experiment's start Albino white male, rabbits were kept at the university of Mosul's dentistry college's animals house for at least two weeks. The evaluation and approval of the research protocol had been

made by the University Of Mosul College Of Dentistry's research ethical committee (UoM. Dent/A.L.55/22). The formula used to determine sample size is as follows :

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

Where: n = sample size required; z (constant) = 1.96 units; r = (precision) = 0.2 units[15]. The resulted number was adjusted, and the final sample size was calculated as the resulted number and the final sample size = (z) \ group. According to this, the calculated sample size for each group was five animals. Datasheets were created for each animal in accordance with the study's protocol, which includes (number, group, weight, anesthetic dosage, magnesium oxide dose, date of orthodontic appliance placement, orthodontic R start date, orthodontic appliance removal date, and sacrifice date). Twenty white male albino rabbits (age range: 6–8 months; weight range: 1100–1450 gm) were divided into four designated metal cages in accordance with the study's design as soon as the sample size was established. They were fed 225g of pellets (Albers® Rabbit 16 percent Animal Feed) daily and kept on a 12:12 light/dark cycle. Every rabbit's weight was checked five days a week during the experiment .To account for potential food type effects on the outcomes, the diet of the entire group was adjusted. Throughout the course of the trial, all rabbits were fed a regular concentrated pellet diet along with leafy greens that were weighed per kg per day. There is always water available. Four groups of five animals each were created by using a straightforward random procedure to divide the animals.

#### *Study design*

The total sample size (20) was divided into 2 main groups.

**A. Control group:** The control group was subdivided into two groups.

1. Negative control group (C-ve): where no magnesium supplementation was taken for four weeks, no orthodontic appliances were used, and distilled water was administered in the same way magnesium was administered to the other groups.
2. Positive control group (C+ve): with orthodontics device given only distilled water for 3 weeks without magnesium intake.

**B. Treatment group** was subdivided into two

(2) groups with the following orthodontic appliances supplemented with MgO:

1. Low dose (LD) group: Low-dose magnesium treatment for three weeks at 40 mg/kg body weightorally [16].
2. High dose (HD) group :received a magnesium dosage of 80 mg/kg for three weeks [17]( high dose with Orthodontic appliances) on the animals' lower incisors, a modified set of fixed orthodontic equipment was placed.

Following two weeks of relaxation, each rabbit underwent three weeks of orthodontic tooth movement. Thus every appliance included two lower central incisor bands of size 000 (Dentaurum, Ispringen, Germany), a sectional arch wire of 0.017 x 0.025 stainless steel, and a continuous nickel titanium open coiled spring (IOS, USA), which was activated to achieve 4.5 mm of spacing and inserted between the bands of two lower incisors to induce a force to the two adjust teeth.

A tension gauge was utilized to determine the force in it prior to the spring being put in. When the springs were in use, they exerted a force of  $40 \pm 2$  g on the distal sides of the treated incisor roots. This put pressure on the treated roots' distal sides and tension on the PDLs of teeth with mesial surface teeth. Before inserting the appliance, ligature wire (Dentaurum, Ispringen, Germany) was kept in position in the holes of the brackets to hold each wire in place. To relax the animal's muscles, a mixture of xylazine (10 mg/kg IM) and ketamine (35 mg/kg IM) was injected. The lower incisor bands of the animal were then cemented using a glass-ionomer cement (TokusoInomer, Tokoyama, Japan). The orthodontic appliance was already activated when the experiment began, and it wasn't activated again while it was running, according to Fig.(1)

#### *Magnesium supplementation*

The magnesium supplement was administered intraorally to the treated group in the form of a 250 mg MgO tablet (21st century health care, AZ 85282, USA). For the LD group, one tablet was mixed with 5 ml of distilled water, with each 1 ml containing 50 mg of magnesium. The magnesium supplement was ordered from 21st CenturyHealthcare in Arizona. The LD group received 0.8 ml once daily at 12 p.m. for a total of three weeks, whereas the HD group received 1.6 ml/kg on a regular schedule.as shown in Fig.(2)



**Fig. 1. Illustration rabbit with orthodontic appliance.**



**Fig. 2. Illustration of study material magnesium oxide.**

#### *Biochemical Assessments*

Where the end of the study duration, the rabbits was sacrificed with an excess of ketamine hydrochloride and blood was collected from the jugular vein. After permitting 5 ml of blood to remain for 30- minutes at room temperature, it was centrifuged at 3000 rpm for twenty minutes to separate the serum from the blood. Serum samples were then extracted, transferred to fresh Eppendorf tubes using a micropipette, and refrigerated at -20 degrees Celsius until analysis Determination of alkaline phosphatase (ALP) activity.

In accordance with the manufacturer's recommendations, the activity of alkaline phosphatase was determined using a colorimetric

test kit. Fuji film Europe dri-chem slide ALP-P - For alkaline phosphatase activity .

#### *Detection of Rabbit Serum calcium and magnesium*

In accordance with the manufacturer's instructions, a colorimetric test kit was also used to determine the serum calcium concentration. European Fujifilm Dri-Chem Slides Ca- PIII For calcium concentration

#### *Detection of Rabbit Serum magnesium*

The serum magnesium concentration was measured using a colorimetric test kit in accordance with the manufacturer's recommendations. Fujifilm Europe dri-chem slide Mg-PIII

*Gene expression analysis:*

Gene expression analysis typically involves the isolation or capture of transcribed RNA within a sample, followed by amplification and subsequent detection and quantitation, its study the occurrence or activity of the formation of a gene product from its coding gene. It is a sensitive indicator of biological activity wherein a changing gene expression pattern is reflected in a change of biological process. Gene expression profiling goes beyond the static information of the genome sequence into a dynamic functional view of an organism's biology and is a widely used approach in research, clinical and pharmaceutical settings to better understand individual genes, gene pathways, or greater gene activity profiles. Gene expression analysis can be achieved through a variety of means, however real-time PCR has risen as the most popularly used approach and the range of products in the Meridian portfolio are well placed to support these studies.

At the end of the study duration no death was happen of the any animals study ,the blood was collected from the jugular vein before scarifying after that Scarifying the rabbits and preparing of the Genomic Assessments. Scarification was performed College of Dentistry, University of Mosul 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, also part of gum beside bone was taken and the periodontal ligament and all were inserted in buffered saline pH (7.4) to prevent its break down.

*Tissue extraction protocol:*

1. Place up to 20 mg of tissue (Bone , gum and periodontal ligament) that has been cut into smaller pieces in a 1.5 ml microcentrifuge tube with 200 µl of Lysis Solution.
2. Fill the sample tube with 20 µl of Proteinase-K-solution (20 mg/ml), proper mixing by vortexing, and then incubate at (56 °C) until the tissue is totally lysed. To make sure that it is distributed evenly during incubation, you

can also put the sample tube in a water bath that is vibrating or on a platform that rocks. The amount of lysis time depends on the kind of tissue that is being treated. Overnight lysis had no impact on the preparation.

3. Rotate the tube downward to clear any droplets from the cap of sample tube.
4. (Optional RNase A treatment) If RNA-free genomic DNA is required, add the 20 µl of RNase A Solution (10 mg/ml, not supplied).
5. After adding 200 µl of the binding solution to the sample tube, thoroughly mix it with a pulse-vortex for 15 seconds.
6. Incubate for 10 minutes at 56 °C. Longer incubation times have no impact on the quantity or caliber of the purified DNA.
7. Include (200 µl) of absolute ethanol and mix thoroughly using a pulse-vortex for (15) seconds. Once you've finished doing this, To remove the drops caught on the lid of the container, quickly spin it.
8. Carefully transfer the lysate into the upper reservoir of the spin column using a 2.0 ml collection tube without soaking the rim.
9. Remove the flow-through and connect the 2.0 ml collecting tube to the spin column after a minute of centrifuging at 13,000 rpm.
10. For 1 minute, centrifuge at 13,000 rpm while adding 500 µl of washing 1 solution u sing a collection tube to connect the spin column: Drain the flowthrough first, then insert the 2.0 ml collection tube into the spin column.1.
11. 500 µl of the Washing 2 Solution should be added. centrifuge for one minute at 13,000 rpm. Remove the flowthrough and put the 2.0 ml collecting tube into the spin column.
12. Dry the spin column by running a further 1 minute of 13,000 rpm centrifugation to remove any remnant ethanol.
13. Use the new 1.5 ml microcentrifuge tube, insert inside the spin column.
14. Pour 100 to 200 µl of the elution buffer solution into the spin column within the micro-centrifuge tube, and then leave it alone for at least a minute.
15. Centrifuge at 13,000 rpm for 1 minute to elute the genomic DNA.

*Primer design for genes (forward and the reverse primer sequence)*

One of the most important aspects of quantitative real-time quantitative Polymerase chain reaction PCR (qPCR) analyses' performance and quality is the design of the primers since effective primer design is essential for accurate

and reliable quantification. To locate possible primers for certain qPCR assays, primer design should follow several criteria. The Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as housekeeping gene. Osteocalcin and VDR gene primers were designed through the use of a well-known website software which is (NCBI).

*Primers design sequence*

Osteocalcin gene primers were designed used NCBI software and synthesized as follow osteocalcin gene forward sequence primer CCGAAACATGGGGTGTGTCT and the reverse sequence

TGCCTTTCTCTGACCCCTACGAPDH forward sequence ACATGCACAGGGTACTTCGA and reverse sequence TTACCCAGCCTTCTCCATG as housekeeping gene, as shown in Fig.(3)

VDR gene primers were designed used NCBI software and synthesized as follow VDR gene forward sequence primer GATGCAGGGCTGTTTATGGG and the reverse sequence GGCCTGCTTGCTGTTCTTAC.

GAPDH forward sequence ACATGCACAGGGTACTTCGA and reverse sequence GGCCTGCTTGCTGTTCTTAC as housekeeping gen, as shown in Fig.(4).

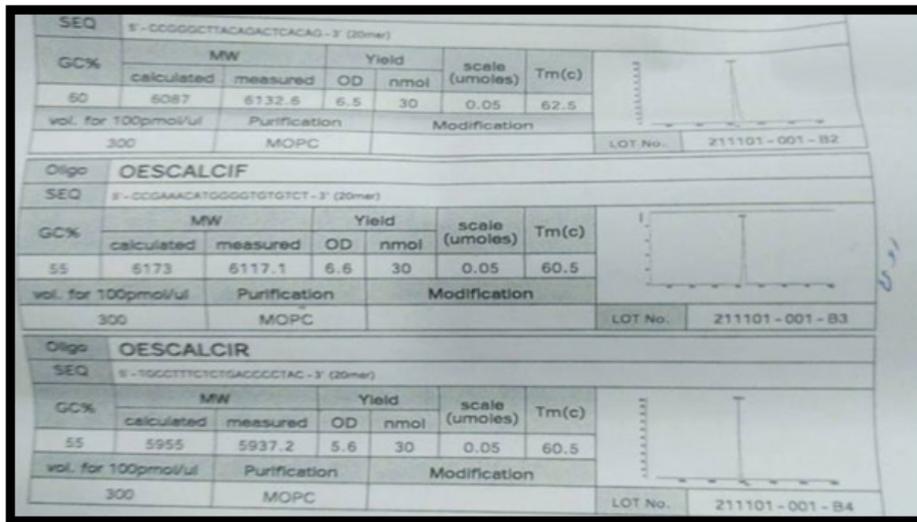


Fig. 3. Osteocalcin gene primers .

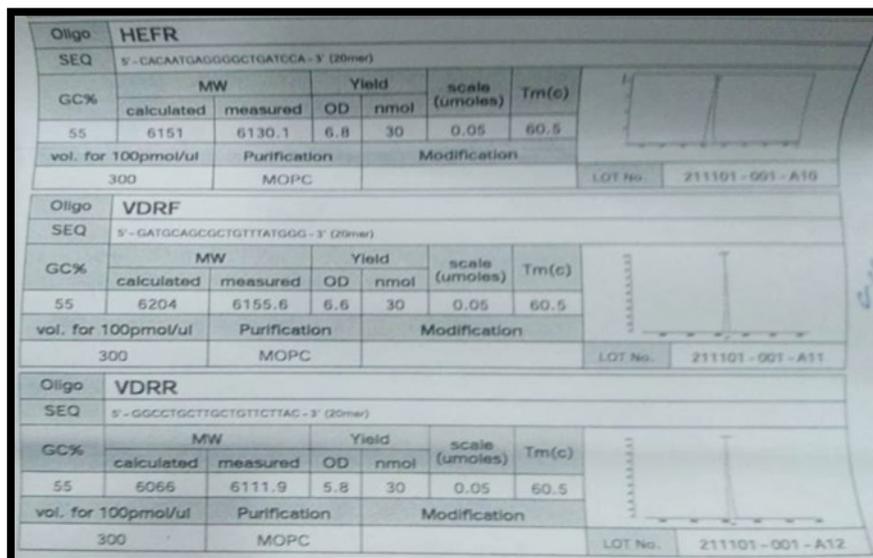


Fig. 4. VDR gene primers .

*GoTaq® qPCR Master Mix:*

The second kit is for quantitative DNA detection which is (GoTaqQpcr Master Mix) from (Promega Corporation USA). The quantitative PCR (qPCR) reagent system GoTaq® qPCR Master Mix (a,b). This system includes a fluorescent DNA-binding dye (BRYT Green® Dye) that binds to double stranded DNA (dsDNA) and exhibits higher fluorescence amplification. All necessary components for qPCR are included in the easy-to-use, stable 2X formulation known as (GoTaq® qPCR Master Mix) except (sample DNA, primers and water).

This formula include GoTaq® Hot Start Polymerase, MgCl<sub>2</sub>, dNTPs, a custom reaction buffer, a proprietary dsDNA-binding dye, and a low concentration of carboxy-X-rhodamine (CXR) reference dye (identical to ROXTM dye) yields the best results in qPCR tests. For use with instruments that need more reference dye than what is in the GoTaq® qPCR Master Mix, a separate bottle of CXR Reference Dye is provided.

*Genomic study*

Includes quantitative measurement of osteocalcin and vitamin D receptors DNA materials. DNA extracted as described above in details from bone tissues at the site of bony defect by using AddPrep Genomic DNA Extraction Kit. The osteocalcin and VDR genomic material determined by (qPCR) by using the (Go-Taq-qPCR master mix) produced by Promega and PCR max Eco machine. Replication reactions of the goal gene and household genes were performed for the samples.  $\Delta\Delta Ct$  calculated for comparison of genes between samples. Replication reactions were done for the genes of of the study and household genes were done for all samples. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping genes were used as a control to calculate the  $\Delta CT$  value.  $\Delta\Delta CT$  calculated for comparison the results of gene expression between samples. The  $\Delta CT$  value calculated for each sample as the difference in CT between the gene of interest and the household gene.  $\Delta\Delta CT$  was measured as the difference between the  $\Delta CT$  values of the study sample and the control sample. The osteocalcin and VDR receptor,s genes in this study expressed as  $\Delta\Delta CT(\text{mean} \pm \text{SD})$ .

$$\begin{aligned}\Delta CT(\text{Sample}) &= CT \text{ ND1gene} - CT \text{ GAPDH} \\ \Delta CT(\text{control}) &= CT \text{ control} - CT \text{ GAPDH} \\ \Delta\Delta CT &= \Delta CT(\text{Sample}) - \Delta CT(\text{control}).\end{aligned}$$

*Statistical analysis*

The descriptive analysis (Mean and Stranded deviation) of the data for the biochemical study ( serum alkaline phosphatase , serum calcium and serum magnesium ) and genomic study (osteocalcin and VDR )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 Tukey 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 were used to detect an intra-class correlation and the reliability of the measurement.

**Results***Biochemical parameters :*

*Level of Serum total Alkaline Phosphatase (ALP), serum Ca+2 and serum Mg+2):*

Statistical analysis by ANOVA test at  $p \leq 0.05$  showed no significant difference of serum Mg+2 level at HD group (54.2±3.53 mg/dl) and LD group (51.75±3.03 mg/dl) in a group treated with magnesium oxide and in compared to control positive group (54.1±3.5 mg/dl) and control negative group (53.5±3.2 mg/dl) as shown in Table (1)

Statistical analysis by ANOVA test at  $p \leq 0.05$  showed no significant difference of serum Ca+2 level at HD group (13.35±0.137 mg/dl) and LD (13±0.514 mg/dl) respectively in and group treated with magnesium oxide and in compared to control positive group (13.16±0.225 mg/dl) and control negative group(13.15±0.05 mg/dl) as shown in Table (1),

Statistical analysis by ANOVA test at  $p \leq 0.05$  showed no significant difference of serum Mg+2 level at HD group (2.93±0.273 mg/dl) and LD group (2.52±0.704 mg/dl) in a group treated with magnesium oxide and in compared to control positive and negative group (2.91±0.248, 3.15±0.05 mg/dl) respectively, as shown in Table (1)

**TABLE 1. Descriptive analysis (Mean and standard deviation) and ANOVA-test comparisons of rabbits total serum alkaline phosphatase(mg/dl), serum calcium and magnesium at the end of the study period.**

P-value	High dose Mean ± SD	Low dose Mean ± SD	Control positive Mean ± SD	Control negative Mean ± SD	Group Serum
<b>0.176</b>	54.2±3.53	51.75±3.03	54.1±3.5	53.5±3.2	Alkaline phosphatase (total)
<b>0.315</b>	13.35±0.13	13±0.514	13.16±0.225	13.15±0.05	Calcium
<b>0.747</b>	2.93±0.273	2.52±0.70	2.91±0.248	3.15±0.05	Magnesium

The mean and standard deviation are often used to summarize the data,

By using one-way variance analysis (ANOVA and Tukey HSD post hoc test).  $P \leq 0.05$  significant variations among groups

### Result of Genomic study

#### A. In gum tissue

##### Osteocalcin gene expression

The results of this work revealed that Osteocalcin with low dose significantly up regulation ( $0.021 \pm 0.002$ ) in contrast to high dose significantly down regulation ( $0.011 \pm 0.004$ ) in comparison to control ( $0.0125 \pm 0.001$ ) at ( $p \leq 0.05$ ). as shown in Fig.(5)

##### VDR gene expression

The results of this work revealed that in gum tissue; VDR low dose ( $0.088 \pm 0.002$ ) and high dose ( $0.0855 \pm 0.0018$ ) no significantly up regulation or down regulation in comparison to control positive ( $0.0847 \pm 0.0004$ ) ( $p > 0.05$ ). as shown in , Fig.(6)

#### B. In the ligament

##### Osteocalcin gene expression

The results of this work revealed that in ligament tissue; Osteocalcin gene expression at LD significantly up regulation ( $0.86 \pm 0.21$ ) in comparison to control ( $0.26 \pm 0.15$ ), high dose significantly down regulation (mean  $\pm$ SD) in comparison to control ( $0.36 \pm 0.12$ ) at ( $p \leq 0.05$ ). as shown in Fig.(7)

##### VDR gene expression

The results of this work revealed that in ligament tissue; VDR low dose ( $0.27 \pm 0.02$ ) and high dose ( $0.27 \pm 0.01$ ) no significantly up regulation or down regulation in comparison to control positive ( $0.27 \pm 0.01$ ) ( $p > 0.05$ ). as shown in Figure( 8)

#### C. In bone tissue

##### Osteocalcin gene expression

The results of this work revealed that

osteocalcin gene expression low dose significantly up regulation ( $3.877 \pm 0.46$ ) in contrast to high dose significantly down regulation ( $2.250 \pm 0.59$ ) in comparison to control ( $3.159 \pm 0.68$ ) ( $p \leq 0.05$ ) as shown in Fig. (9)

##### VDR gene expression

The results of this work revealed that in bone tissue; VDR gene expression low dose significantly up regulation ( $5.87 \pm 0.38$ ) in contrast to high dose significantly down regulation ( $4.25 \pm 0.66$ ) in comparison to control ( $4.48 \pm 0.82$ ) ( $p \leq 0.05$ ) as shown in Fig. (10)

### Discussion

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, since, they were the most commonly used species in bone studies[18]. The goal of the select seven days of orthodontic tooth movement was to promote rapid bone turnover. In addition, previous studies[19], have observed genomic changes that occur when animal undergoes orthodontic treatment 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, Orthodontic appliance for lower incisors was less distressing for the animals than other appliances were if they were constructed posteriorly between the first molars and the lower incisors

[19]. The males' rabbit was recruited in this study in order to exclude hormonal 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. 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 [20]

In this study serum  $\text{Ca}^{+2}$  showed no significant difference of serum  $\text{Ca}^{+2}$  level with the administration of high dose and low dose MgO and in comparison to control positive group and control negative group. In a study by Rodríguez-Ortiz et al show that Mg was able to reduce PTH only if parathyroid glands were exposed to moderately low  $\text{Ca}^{+2}$  concentrations; with normal–high  $\text{Ca}^{+2}$  concentrations, the effect of Mg on PTH inhibition was minor or absent. This might explain the no significant effect of magnesium supplement in the PTH regulation of serum  $\text{Ca}^{+2}$  [21].

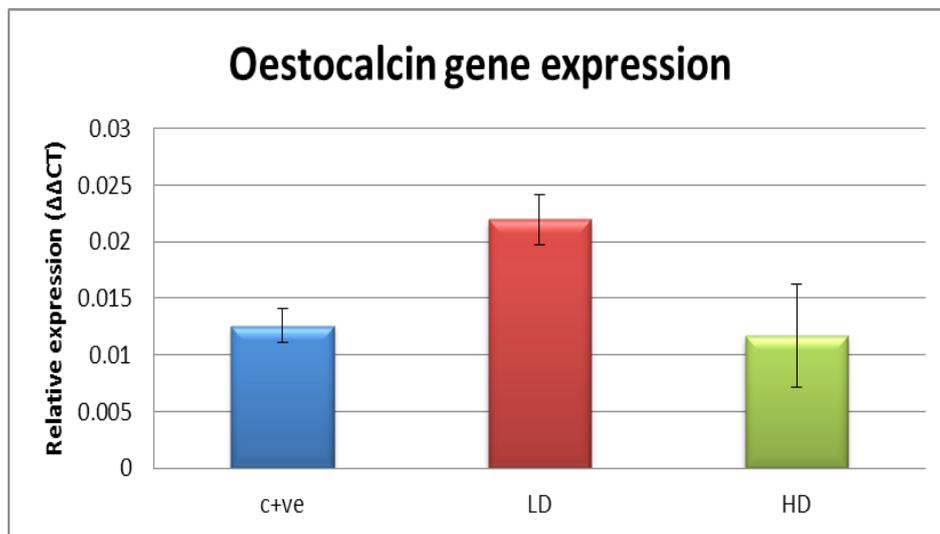


Fig. 5. Osteocalcin gene expression mean founded in comparison of the study groups, founded in the gums, at end of study period .

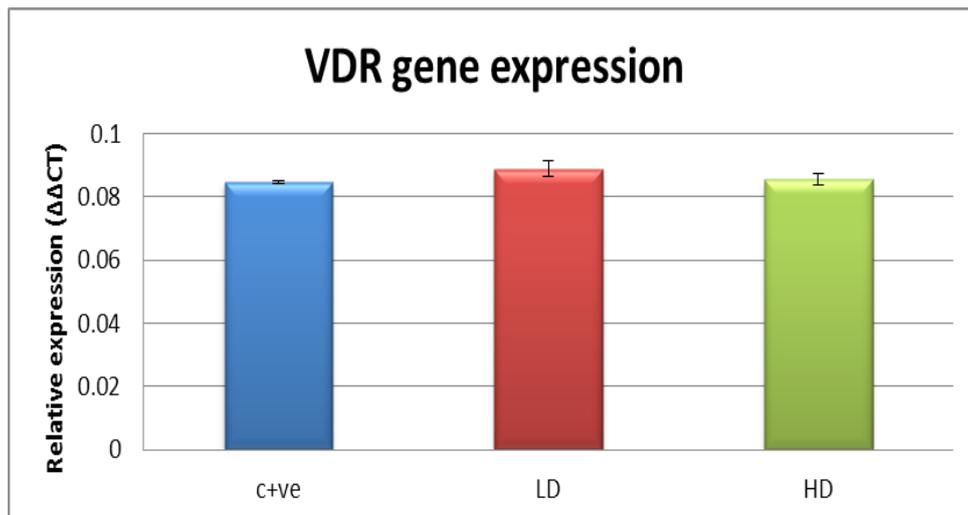


Fig. 6. The comparing of VDR gene expression mean founded in comparison of the study groups, founded in the gums, at end of study period.

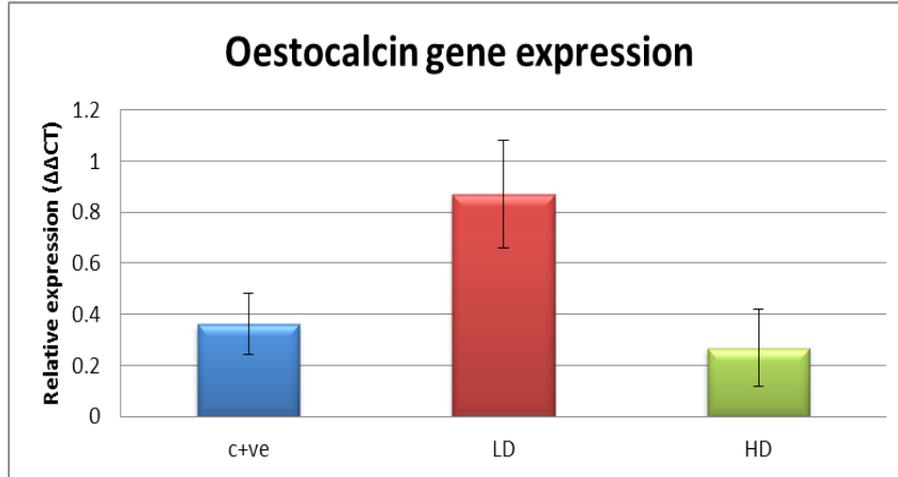


Fig. 7. The comparing of osteocalcin gene expression mean founded in comparison of the study groups, at end of study period founded in the ligament tissue.

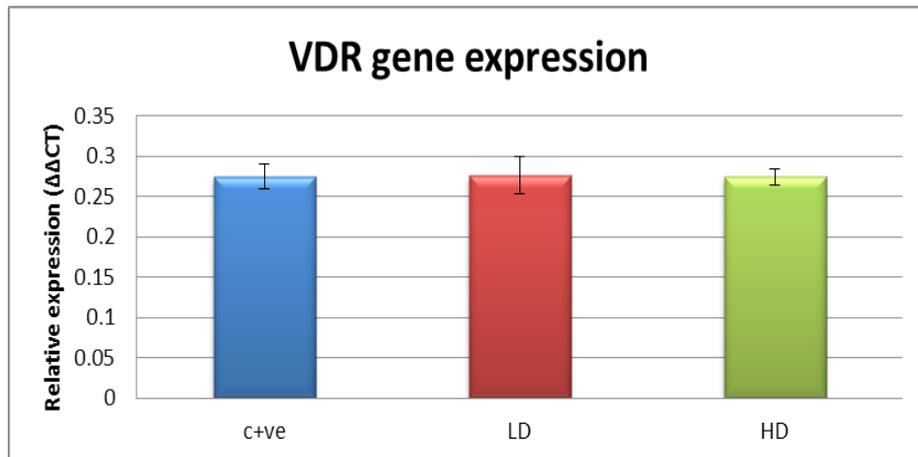


Fig. 8. The comparing of VDR gene expression mean in comparison of the study groups, at end of study period founded in the ligament tissue.

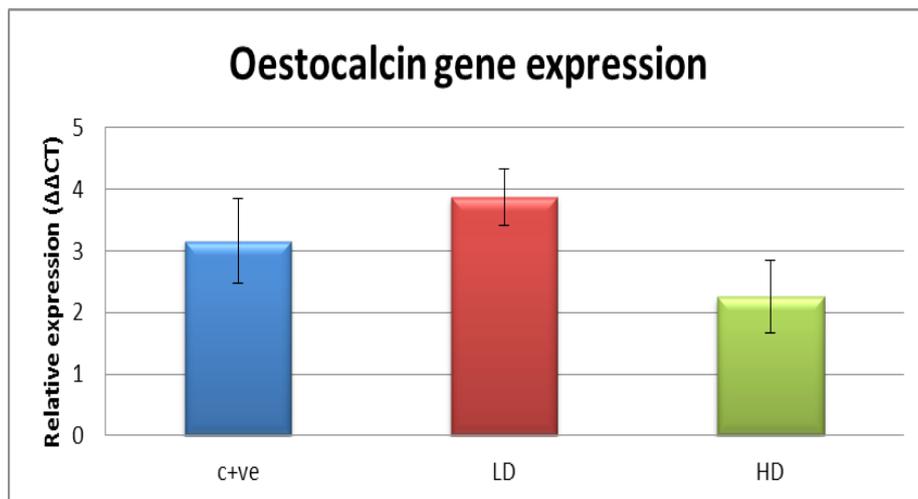


Fig. 9. The comparing of osteocalcin gene expression mean founded in comparison of the study groups, at end of study period founded in the bone tissue.

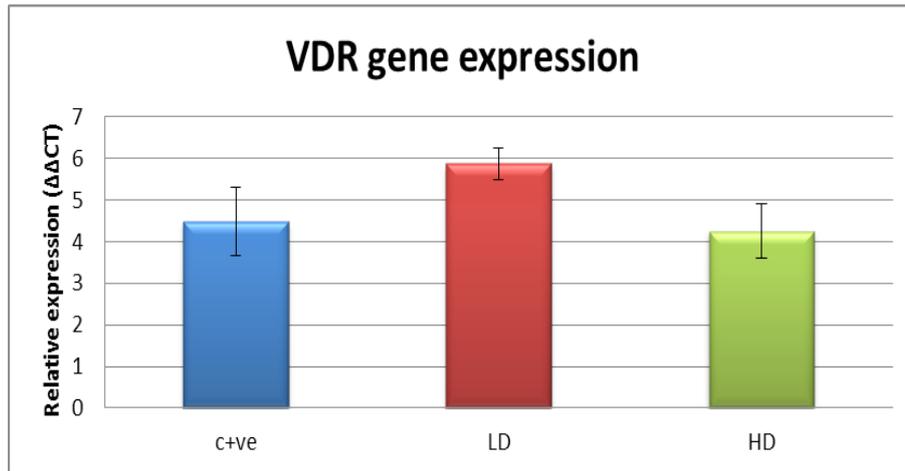


Fig. 10. The comparing of VDR gene expression mean founded in comparison of the study groups, at end of study period founded in the bone tissue.

A study by Monalisha et al. crucial factor to the success of orthodontic treatment is a proper vitamins and minerals most importantly Mg+2 & Ca+2. Orthodontic appliance attachment breakage is frequent orthodontic problem, which can be prevented by a proper diet and mineral supplementation. A soft, nutritious diet including all the necessary elements is vital for preserving good orthodontic repair [22].

Castiglioni et al. found that Magnesium deficiency interferes with the parathyroid hormone. Magnesium overdose increases bone mineral density in other word it decrease the Ca+2 in circulation by depositing it in to bones leading to reduce S.Ca. [5]. However, Novack et al. express that the bone resorption by the action of osteoclast in orthodontics circulatory Ca+2 although its action might not enough to significantly increase S.Ca owing to smaller bone resorption (only teeth roots)[23]. These opposite actions might the cause of non-significant change in serum Ca+2 in our study.

Regarding serum Mg+2 showed non-significant difference of serum Mg+2 level high dose and low dose group in a group treated with magnesium oxide and in comparison to control positive group and control negative group. In healthy subject there is a balance system keeps the magnesium with in normal value. Mg+2 cannot be stored but only retained for current needs; a higher intestinal absorption is usually followed by a higher renal excretion of the mineral under normal condition

Nakamura et al. conducted two experiments

where the effects of the artificial Mineral Water and the Mg+2 concentration on Mg+2 absorption is checked. Results suggest that the Mg+2 amount and concentration do not affect Mg+2 absorption but that Mg+2 absorption decreases when the amount of Mg+2 intake is increased. This suggest presence of intestinal absorption regulation for Mg intake of higher concentration[24].

Same as serum magnesium level a study by Arash et al. found that no significant change in the level of magnesium in saliva after orthodontic treatment[25]. In a study by Zhang et al., found that Magnesium supplementation is vital for bone mineralization, however high level of magnesium probably exceed the renal excretion ability (in case of renal diseases) may inhibit bone mineralization, thus While high Mg+2 hinders the osteogenesis of bone While appropriate amounts of Mg+2 can enhance mineralization[26].

Regarding ALP showed non-significant difference of serum Mg+2 level in both high dose and low dose magnesium oxide treated groups in comparison to control positive and control negative groups

Although the differences between the groups isn't significant, Both groups with higher Mg dose and the group supportively with higher inflammation level (control positive group) showed higher ALP and even close result is indicating the relationship between ALP and Mg supplement level and degree of inflammation.

Magnesium is one of the alkaline phosphatase (ALP) cofactor. The Level of

magnesium affect ALP activity, increased ALP activity will indicate the level of inflammation or trauma in periodontal tissue or when there is damage to the bone supporting the teeth[27].

Regarding Osteocalcin the results of this work revealed that in gum tissue; Osteocalcin is significantly up regulated in low dose Mg group in contrast to high does which are significantly down regulated in comparison to control ( $p < 0.01$ ). While in ligament tissue; Osteocalcin with low does mg significantly up regulated in comparison to control , high does significantly down regulated in comparison to control ( $p < 0.01$ ). Same is for bone tissue; osteocalcin gene expression in low does magnesium group significantly up regulated in contrast to high does group that significantly down regulated in comparison to control ( $p < 0.01$ ).

Bone-remodeling cycle is the cross-talk between osteoblasts and osteoclasts and regulated by signals most important one is VD3 which act via activating the expression of osteocalcin . Osteocalcin is secreted solely by osteoblasts and works in conjunction with matrix-GLA proteins to aid in teeth re-mineralization[28]. Mammoli *et al.* found that high Mg levels decrease the osteoblast differentiation of bMSCs (osteocalcin) induced by an osteogenic cocktail containing VD3 were significantly down regulated in response to high Mg+2 exposure. This findings highlight that high Mg levels can result in detrimental effects for bone not only through inhibition of osteoblast differentiation and function, but also through a parallel increase of osteoclast activity [29].

Leidi *et al.* explain that Mg+2 alloys, whose property profile closely resembles that of human bone, were used to create degradable osteosynthesis devices. As Mg+2 alloy degrades, the local concentration of Mg+2 in bone increases. It is well known that high extracellular Mg+2 inhibits the activity of human osteoblasts *in vitro*. [30] . In another study, Kargozar *et al.*, who investigated the osteogenic activity of magnesium (Mg)-doped multicomponent bioactive glass, found that the immunostaining technique detected the production of osteogenic protein markers, including osteocalcin, 12 weeks after implantation. Compared to the control group, osteogenic markers were expressed at a higher level in the holes that were filled with scaffolds that were doped with Mg. This shows that osteogenic differentiation and bone metabolism were increased [31].

Regarding vitamin D receptor expression As the results of this work revealed that in gum and periodontal ligament tissue; low doses and high doses groups of MgO showed no significantly VDR expression in comparison to control as the ( $p > 0.05$ ).

And the results of in bone tissue; low doses of MgO significantly up regulate VDR gene expression , in contrast to high does MgO which significantly down regulate it in comparison to control were ( $p < 0.05$ ).

In a study by Rodríguez-Ortiz *et al.* show that Mg modulates the function of parathyroid glands through up regulation of the key cellular receptors CaR, VDR and the FGF23/Klotho system [21]. Another study by KÜchler *et al.* found that orthodontic compressive forces and of vit D3 seem to regulate the expression of vitamin-D-related genes in periodontal ligament fibroblasts in the context of orthodontic strain. Following the application of orthodontic forces [32], the resorptive and bone-formation phases at the alveolar process comprise bone remodeling. Vitamin D receptor polymorphisms and periodontitis and bone metabolism have both been linked to each other. Zhang *et al.* found that VDR gene polymorphisms is one of the causes for mineral disturbance. Polymorphism of VDR gene in tissue results in variation in vitamin D receptors expression in the tissue including bone, parathyroid gland and others. Thus may affect the cementum and surrounding alveolar bone apposition. VDR may function as locally to suppress osteoblast formation and bone growth[33].

Periodontal remodeling occurs continuously during tooth movement in orthodontics, and alveolar bone and cementum are resorbed under pressure and deposited under tension. The retention plays a crucial part in the long-term success of orthodontic therapy. It is crucial to look for an efficient way to quicken the remodeling of the alveolar and cementum bones following orthodontic and periodontal surgery. Alveolar bone remodeling has reportedly been improved by numerous studies. One study found that Additionally, it has been shown that VDR ligands have anabolic effects on bones.

In a study, Kasuma *et al.* established in a study that there is an association between the levels of alkaline phosphatase and magnesium in patients with periodontal disease. The greater

the inflammatory rate, the greater the ALP elevations. A strong correlation exists between ALP and magnesium levels. Magnesium works as an activator at optimal amounts, while at larger concentrations it becomes an inhibitor. Since both magnesium and zinc ions can bind to the same site, it is plausible that extra magnesium ions displace zinc ions from the catalytic site. The greater magnesium consumption, the less ALP activity there will be. Thus, periodontal disease progression also diminishes. Magnesium supplements might be prescribed by a practitioner as a suitable supplementary treatment to reduce periodontal inflammation. Thus, there is a link between alkaline phosphatase levels and magnesium levels and periodontal disease. Increasing the pace of inflammation will raise the ALP level[34].

However, in a study by Meisel et al., found that a reduction in ALP activity will occur after a decrease in magnesium. Magnesium loss may affect bone supporting teeth over time it disrupts bone remodeling and turnover which leads to aberrant bone growth. Supplementing with nutritional magnesium may enhance periodontal health [35].

In summation, magnesium serves as an activator at ideal concentrations but turns inhibitory at higher concentrations. Maintaining the ideal serum level of Mg<sup>2+</sup> is crucial for magnesium action[36].

#### *Clinical implication*

According to studies, using magnesium supplements at the recommended dosages is economical, generally safe, and well tolerated. It has been proposed that magnesium supplementation may be beneficial for preventing and reducing root resorption during orthodontic tooth movement therapy.

#### **Conclusion**

This study was performed to elucidate the effect of orally administered magnesium oxide bone formation in rabbits with orthodontic appliance. In this study, MgO was administered systemically for four weeks during an orthodontic teeth movement, only Low dose have significant increased the level of bone formation markers such as the gene expression of both osteocalcin and vitamin D receptors in compared with high dose and control group.

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#### *Conflicts of interest*

The authors declared no competing interests.

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## تقييم التأثير الجهازي للمغنيسيوم او كسايد على التعبير الجيني للاوستيوكالسين ومستقبلات فيتامين د للأرانب مع حركة الأسنان التقويمية

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**الخلفية:** العظم نظام ديناميكي يخضع لعملية إعادة تشكيل مستمرة يستجيب لكل المحفزات الخارجية والاستقلابية. لإعادة تشكيل العظام ، لتطوير وتسريع حركة تقويم الأسنان. المغنيسيوم أحد العناصر الرئيسية للإنسان. له عدد من الخصائص خاصة في نمو العظام. **هدف الدراسة:** تقييم التأثير الجهازي أكسيد المغنيسيوم على تكوين العظام في الأرانب مع حركة الأسنان التقويمية المواد وطرق العمل : وقسمت إلى أربعة مجاميع من خمسة لكل منها: اثنان وتزن بين (1100-1450) جرام. **المواد وطرق العمل :** وقسمت إلى أربعة مجاميع من خمسة لكل منها: اثنان ضابطة (إيجابية وسلبية) واثنان تجريبيان (جرعة منخفضة وجرعة عالية). خلال فترة تقويم الأسنان ، أعطيت الحيوانات في المجموعة التجريبية الأولى جرعة منخفضة من 40 مجم / كجم وأعطيت المجموعة التجريبية الأخرى جرعة عالية 80 ملجم / كجم وزن حي من MgO يومياً لمدة ثلاثة اسابيع . تم تجهيز القواطع المركزية السفلية للأرانب بأجهزة تقويم ثابتة. تم قتلها في نهاية فترة الدراسة لجميع المجموعات تم جمع عينة الدم وعزل الفك السفلي وعزل عينات العظام المقطوعة من موقع الأسنان المتحركة واللثة والأربطة اللثوية ، ووضعت في محلول ملحي الفوسفات ليتم تقييمها ب ال PCR الكمي تم تحليل البيانات احصائيا باستخدام ANOVA و Tukey عند مستوى  $p \leq 0.05$  لفروق ذات دلالة احصائية. **النتيجة:** لم تظهر النتيجة أي تغيرات معنوية عند  $p \leq 0.05$  في مصل  $Ca^{+2}$  الدم و  $Mg^{+2}$  والالكالين فوسفاتيز المجموعات التجريبية MgO ومقارنة والمجموعات الضابطة ، وأظهرت الدراسة الجينية زيادة في التعبير الجيني الكمي لكل من الأوستيوكالسين ومستقبلات فيتامين د (للعظام واللثة والرباط المحيطي) في الجرعة المنخفضة المعالجة بالمغنيسيوم مقارنة بالمجموعة الضابطة ومجموعة الجرعات العالية في نهاية فترات الدراسة. **الخلاصة:** الجرعة المنخفضة من المغنيسيوم تزيد من تكوين العظام وتجديدها

**الكلمات المفتاحية :** مغنيسيوم او كسايد ، التعبير الجيني ، تكوين العظم ، تقويم الاسنان.