

# The Effect of Vitamin D<sub>3</sub> on the Eruption of Teeth

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## ABSTRACT

**Aims of the Study:** Evaluation of the effects of vitamin D<sub>3</sub> on the eruption of teeth, on BAP, TRACP, MMP1, MMP3 concentration and on the number of osteoblasts, osteoclasts, fibroblasts, blood vessels, and on new bone formation.

**Materials and Methods:** The samples consisted of 24 females new Zealand white rabbits. The eruption rates were measured using Digital Caliper in parts of millimeters. **Experimental groups included:** Vitamin D group consisted of twelve rabbits which were randomly divided into three subgroups of four rabbits received vitamin D<sub>3</sub> and Control group Consisted of 12 rabbits which received no treatment. They were randomly divided into three subgroups of four rabbits each Histological evaluation hematoxylin and eosin (HandE) stain was used. **Biochemical assay:** (ELISA) biochemical tests were employed. These tests were: Rabbit Bone Specific Alkaline Phosphatase (BAP), Rabbit Tartrate Resistant Acid Phosphatase (TRACP), Matrix metalloproteinase 1 (MMP1) and Matrix metalloproteinase 3 (MMP3).

**Results :** Showed that vitamin D accelerates eruption and caused a significant elevation of BAP level when compared to control with a rise of about seven folds in (D<sub>3</sub>w1) to ten folds in (D<sub>3</sub>w2). Histomorphometrically vitamin D<sub>3</sub> increased BTT, number of osteoblasts and area of new bone formation when compared to control.

**Conclusions:** The systemic administration of vitamin D<sub>3</sub> enhanced eruption rate. This acceleration could be attributed to the positive effects of vitamin D<sub>3</sub> on bone.

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## INTRODUCTION

Tooth eruption is defined as the movement of a tooth from its site of development within the jaws to its position of function within the oral cavity<sup>(1)</sup>. Vitamin D is of large importance for homeostasis of calcium and bone metabolism. In the first place, vitamin D is responsible for maintaining the extracellular calcium concentrations by controlling absorption of calcium and by direct effects on bone and on parathyroid hormone (PTH) secretion<sup>(2)</sup>. The rate of formation or degradation of the bone matrix can be assessed by measuring the enzymatic activity related to the bone forming or resorbing cells. Bone matrix components are released into the circulation, either by the osteoblasts or by the osteoclasts<sup>(3)</sup>. Tartrate-resistant Acid Phosphatase 5b (TRACP 5b) is a unique bone resorption marker, because it is the only marker released from resorbing osteoclasts, instead of being a bone matrix degradation product. In circulation TRACP exists in two isoforms named TRACP 5a and 5b, of which TRACP 5b is specifically derived from osteoclasts, and TRACP 5a from activated macrophages and dendritic cells<sup>(4,5)</sup>.

It is generally accepted that alkaline phosphatase (ALP) plays a role in the process of hard tissue formation. High levels of the enzyme activity have been reported to be present in active sites of ossification and during odontogenesis there is a gradient of ALP activity coincident with enamel and dentine matrix synthesis and during calcification<sup>(6,7)</sup>. The potential role of proteases in the dental follicle during tooth eruption may include degradation of capillary basement membrane sites, thus facilitating entry of osteoclast precursor cells into follicle from blood also degradation of cell-matrix contact regions leading to reorganization of cell-cell and cell-matrix interactions necessary for periodontal ligament development<sup>(8)</sup>.

**Aims of the Study:** Evaluation of the effects of vitamin D<sub>3</sub> on the eruption of teeth, Studying the effects of vitamin D<sub>3</sub> on Bone Alkaline Phosphatase (BAP), Tartrate-resistant Acid Phosphatase (TRACP), Matrix Metalloproteinase 1 (MMP1) and Matrix Metalloproteinase (MMP3) concentrations, and investigating the effects of vitamin D<sub>3</sub> on the number of osteoblasts, osteoclasts, fibroblasts, blood vessels, and on new bone formation.

## MATERIALS AND METHODS

The samples consisted of 24 females new Zealand white rabbits which were obtained from the local farms, of an average weight 600gm (500gm-750gm) housed in an iron cages and fed with vegetables, corn, and grains and supplied with tap water *ad libitum*. They were randomly divided into two groups. The scientific committee of college of dentistry /University of Mosul approved our protocols. Prior to each treatment, the rabbits were anaesthetized with xylazine 0.2 cc/100 g body weight (interchemie, Holland 20mg/ml) and ketamine 0.2 cc/100 g body weight (Hamlen pharmaceuticals gmbh, Germany 50mg/ml injection) combination.

The eruption rates were measured by marking the right lower incisor of each rabbit by drilling a small hole on the labiodistal side of the tooth near the gingival margin with small round carbide bur in low speed hand piece. A new mark was drilled at every measurement and the distance between the gingival margin and the mark was measured using Digital Caliper in parts of millimeters. The eruption rates were measured twice at three days interval before starting to give the different agents used in this study to provide a record for normal eruption(9).

### Experimental groups

#### A-Vitamin D group

Consisted of twelve rabbits which were randomly divided into three subgroups four rabbits for each group:

##### 1-Vitamin D Week One (Dw1)

Received 60000iu/kg vitamin D<sub>3</sub> I.M. single injection (DEVIT-3I.M./oral DEVA HOLDING A.S. Turkey 300000iu/ml) at day 0. The eruption rates were examined at day 3 and day 7. The collection of blood and animal scarification was made on day 7.

##### 2-Vitamin D Week Two (Dw2)

Received 60000 iu/kg vitamin D<sub>3</sub> I.M. twice in day 0 and day 7. The eruption rates were recorded at days 3,7,10 and 14. The collection of blood and animals scarification was made on day 14.

##### 3-Vitamin D Week Three (Dw3)

Received three separate doses of 60000iu/kg vitamin D<sub>3</sub> I.M. at days 0, 7 and 14. The eruption rates were measured at days 3,7,10,14,17 and 21. The collection of blood and animals scarification was made on day 21.

#### B-Control group

Consisted of 12 rabbits which received no treatment. They were randomly divided into three subgroups:

##### 1- Control Week One (Cw1)

The eruption rates were measured on days 3, 7. the collection of blood and animals' scarification was made on day 7.

##### 2-Control Week Two (Cw2)

The eruption rates were measured on days 3, 7, 10, 14. The collection of blood and animals scarification was made on day 14.

##### 3-Control Week Three (Cw3)

The eruption rates were measured on days 3,7,10,14, 17 and day 21. The collection of blood and animals scarification was made on day 21.

### Histological Evaluation:

The lower jaw of each rabbit was dissected from the head. The anterior teeth with their supporting bone were cut (the cutting was made anterior to the premolars bilaterally). They were then fixed in 10% buffered formalin solution after that they were decalcified in 10% nitric acid. After decalcification they were embedded in paraffin blocks in such a way that the sectioning is made parallel to the long axis of the tooth starting from the inferior border of the jaw. The sectioning was made in 5 micron thickness, stained with hematoxylin and eosin (HandE) stain.

The teeth together with the periodontium and supporting bone were examined under light microscope 450x by a specialist in histology. Bone osteoblasts, osteoclasts, osteocytes, bone trabeculae thickness, blood vessels and areas of new bone formation were examined and recorded in six bone areas around the right lower incisor (mesial and distal) in the coronal, middle and apical levels of the tooth. Recording the number of osteoblasts, number of osteoclasts, number of osteocytes, number of blood vessels and bone trabeculae thickness was performed directly on the microscope while measuring the percentage of area of new bone formation was done on by Image J computer program by measuring the percentage of number of peckcells of new bone to the number of peckcells of the examined area.

#### Biochemical assay:

The blood samples were collected into tubes and after settling of about half an hour they were centrifuged for 15 minutes (3000 rpm).

The extracted serum sample was stored in -20 C° until they were assayed. They were allowed to melt at room temperature and four Enzyme-Linked Immuno Sorbent Assay (ELISA) biochemical tests (Biosource USA) were performed in accordance with the manufacturer instructions. These tests were: Rabbit Bone Specific Alkaline Phosphatase (BAP), Rabbit Tartrate Resistant Acid Phosphatase (TRACP), Matrix metalloproteinase 1 (MMP1), and Matrix metalloproteinase 3 (MMP3).

#### STATISTICAL ANALYSIS

The eruption rate clinical measurements, BAP, TRACP, MMP1 and MMP3 concentration in blood and the histological finding including osteoblasts and osteoclasts, bone trabeculae thickness, blood vessels and areas of new bone formation were statistically analyzed using SPSS program: Descriptive statistics and Independent Sample T-Test was performed between the two groups. Values of  $p \leq 0.05$  were considered statistically significant.

#### RESULTS

The eruption rates demonstrated in (Table1) showed that the highest mean value was noticed in (D<sub>3</sub>w3) subgroup which also recorded the highest maximum value. On the other hand the lowest mean was seen in Control week two. Independent sample T- test showed a significantly higher eruption rates in vitamin D subgroups when compared to Control subgroups in week one, week two and week three periods. BAP showed an obvious rise in vitamin D subgroups when compared to control. Vitamin D week two (VD<sub>3</sub>w2) recorded the highest mean value followed by Vitamin D week three (D<sub>3</sub>w2) and Vitamin D week one (D<sub>3</sub>w1) subgroups respectively.

The standard error was the highest in (D<sub>3</sub>w3) subgroup (Table 2). The same was noticed in TRACP where the highest mean value was recorded in (D<sub>3</sub>w2) followed by (D<sub>3</sub>w1), control and (D<sub>3</sub>w3) subgroups respectively and the highest standard error was seen in (D<sub>3</sub>w1). Also (D<sub>3</sub>w2) showed the highest mean value and the highest standard error of MMP1 on the other hand (D<sub>3</sub>w3) demonstrated the lowest mean value. On the other hand the slight rise seen in MMP3 mean value in (D<sub>3</sub>w1) subgroup compared to control was followed by a decline in (D<sub>3</sub>w3) and (D<sub>3</sub>w2) respectively (Table 2). Independent Sample T-Test Between Control and vitamin D subgroups of MMP1, MMP3, BAP and TRACP concentrations is shown in (Table3), no significant difference was noticed except in BAP concentration where (D<sub>3</sub>w2) recorded a significantly higher value than control.

Histological evaluation was performed in 40x and 100x (figures 1-8). Descriptive statistics of blood vessels, bone trabeculae thickness (BTT), Osteoblasts, Osteocytes and percentage of area of new bone formation (%ANBF) is shown in (Table 4). Blood vessels number was lower in (D<sub>3</sub>w2) and (D<sub>3</sub>w3) subgroups than in control while the highest value was seen in (D<sub>3</sub>w1) which also recorded the highest standard error. Bone Trabeculae Thickness (BTT) showed increased mean value in vitamin D subgroups compared to control with the highest thickness was recorded in (D<sub>3</sub>w3) subgroup and then by (D<sub>3</sub>w2) and (D<sub>3</sub>w1) subgroups respectively. Osteoblasts number raised in all Vitamin D subgroups over control. The highest elevation was seen in (D<sub>3</sub>w2) followed by (D<sub>3</sub>w3) and then (D<sub>3</sub>w1). The highest range, maximum value and standard error were seen in (D<sub>3</sub>w2). Bone Osteocytes was higher in (D<sub>3</sub>w1) followed by (D<sub>3</sub>w3) and then (C) subgroups. While the lowest mean value was seen in (D<sub>3</sub>w2). The percentage of area of new bone formation (% A.N.B.F.) showed a high elevation in vitamin D subgroups in comparison to Control with the highest value recorded in (D<sub>3</sub>w3) followed by (D<sub>3</sub>w2) and (D<sub>3</sub>w1) respectively.

Independent Sample T- Test Between Control and Vitamin D Subgroups of Blood Vessels, BTT, Osteoblasts, Osteocytes and Percentage of Area of New Bone Formation is demonstrated in (Table 5). Blood vessels were significantly lower in (D<sub>3</sub>w2) and (D<sub>3</sub>w3) subgroups when compared to control. While BTT was significantly higher in (D<sub>3</sub>w3). On the other hand osteoblasts showed a significantly higher mean value (D<sub>3</sub>w2) than (C). Osteocytes did not record a significant difference between vitamin D and Control. On the contrary, very high significant differences were seen in the percentage of area of new bone formation in vitamin D subgroups in comparison with control.

## DISCUSSION

Tooth eruption has been studied on bases of proposed mechanisms of eruption, associated bony changes, cellular participation, enzymes and molecules possibly involved, timing of eruption and the effect of systemic, genetic and local factors on tooth eruption. The effect of different agents on the rate of eruption had been discussed.

Variation in the normal eruption of teeth is a common finding, but significant deviation from established norms should alert the clinician to take some diagnostic procedures in order to evaluate patient health and development<sup>(10)</sup>. Eruption rates showed a significant increase in vitamin D group when compared to control and the acceleration was noticed from week one.

### The Effect of Vitamin D on BAP, TRACP, MMP1 And MMP3 :

Vitamin D has an anabolic effect on bone metabolism and has a regulatory function in bone formation and mineralization.

In vitamin D group a sharp elevation was noticed in the level of serum alkaline phosphatase concentration when compared to control and this comes in agreement with the findings of Trautvetter *et al* (2014)<sup>(11)</sup> who found an increase of plasma alkaline phosphatase concentration after four weeks vitamin D supplementation, but it disagrees with the findings of Viljakainen *et al* (2009)<sup>(12)</sup> who found that the level of alkaline phosphatase had decreased with the chronic administration of vitamin D. This can be explained by the fact that the chronic administration of vitamin D can increase bone formation but it would reduce mineralization, a finding which was confirmed by Gallagher *et al*, 2013<sup>(13)</sup> who stated that high concentration of 1, 25 (OH)<sub>2</sub> D<sub>3</sub> inhibits mineralization.

TRACP levels had not been affected by the administration of vitamin D and this comes in agreement with the findings of Viljakainen *et al* (2009)<sup>(12)</sup> who found no effect of Vitamin D<sub>3</sub> supplementation on TRACP level in healthy men. However it disagrees with those of Bonjour *et al* (2012)<sup>(14)</sup> who concluded that the consumption of vitamin D and calcium fortified dairy products reduce the serum concentration of TRACP in healthy post-menopausal women.

Vitamin D has been proposed to have a regulatory effect on MMPs; however, controversial results had been obtained in this issue. Kobayashi *et al* (2005)<sup>(15)</sup> had found a suppressive effect of Vitamin D<sub>3</sub> on the production of MMP1 and MMP3 from cholesteatoma keratinocyte in vitro. While Tetlow and Woolley (1999)<sup>(16)</sup> had found that MMP1 and MMP3 production by rheumatoid synovial fibroblast in monolayer culture was not affected by the treatment with vitamin D. On the other hand, Lee *et al* (2013)<sup>(17)</sup> noticed a significant increase in MMP1 and MMP3 release from cultured sebocyte after treatment with vitamin D.

### Histological Evaluation

A direct effect of vitamin D on bone is logical as vitamin D receptor (DR) is present in osteoblasts and precursors of the bone resorbing cells (osteoclasts) and in mature osteoclasts in addition they are present in osteocyte<sup>(18,19,20)</sup>.

Vitamin D<sub>3</sub> plays a major role in the regulation of mineral homeostasis and effects on bone metabolism. It plays a role in the regulation of early stage of human osteoblast differentiation in human bone marrow, stromal cell cultures and clonal cell lines derived from human trabecular bone. It has direct effects on osteoblastic function and can modulate osteoblastic proliferation and osteoblast production of type I collagen, ALP and osteocalcin<sup>(21,22)</sup>.

On osteoclast the administration of high doses of vitamin D<sub>3</sub> will cause an immediate increase in osteoclastic number by enhancing the monocytic differentiation of immature hematopoietic cells and subsequent commitment of monocyte cells into preosteoclast and therefore cause an immediate bone resorption this effect would be counteracted by parathyroid hormone (PTH) which would suppress the osteoclastic activity and the chronic administration of vitamin D<sub>3</sub> would reduce bone resorption<sup>(23)</sup>.

In this study the diminished number of osteoclast after the administration of vitamin D comes in agreement with the findings of Boyce and Weisbrode (1985)<sup>(24)</sup> who found that the injection of high doses of vitamin D<sub>3</sub> increased osteoclast number in tibial cancellous bone on day one but bone resorption was suppressed by day six and also comes in agreement with the finding of Erben *et al* (1994)<sup>(25)</sup> who found that the administration of high doses of vitamin D<sub>3</sub> to rat suppressed histomorphometric parameters of bone resorption four days after discontinuation of vitamin D<sub>3</sub> and this is explained by the action of PTH which tend to counterbalance the action of vitamin D<sub>3</sub>.

Blood vessels number was significantly reduced in Dw2 and Dw3 subgroups when compared to control group which comes in agreement with the findings of Godoy *et al* (2013)<sup>(26)</sup> who stated that systemic changes in vitamin D drastically affect blood flow and vascularity. A significant increase in the number of osteoblasts was recorded in Dw2 subgroup when compared to C group while in Dw1 and Dw3 subgroups. The effect of vitamin D on the proliferation of

osteoblasts showed a controversial results varying from stimulation<sup>(27,28)</sup> to no effect Taira et al (2003)<sup>(29)</sup> or an inhibitory effect<sup>(28,30,31)</sup> who showed that high doses can reduce proliferation of osteoblasts and low doses increased osteoblast. Different species employed in these studies may be possible causes for these varying results.

Bone trabeculae thickness in vitamin D subgroups recorded an increase when compared to control and this rise was significant in Dw<sub>3</sub> subgroup and this comes in agreement with the findings of Gardiner *et al* (2000)<sup>(32)</sup> who indicated bone anabolic effect of D<sub>3</sub> by demonstrating a 20% increased bone trabeculae volume and also agrees with the findings of Erben *et al* (1997)<sup>(33)</sup> with short treatment and those of with chronic treatment with D. But it disagrees with the conclusions of Amling *et al* (1999)<sup>(34)</sup> who suggested a negative effect of D on trabecular development in addition to Amling<sup>(34)</sup>, Smith *et al* (2000)<sup>(35)</sup>, Hoenderop et al (2003)<sup>(36)</sup> and St Arnaud et al (2000)<sup>(37)</sup> also reported a negative effect of D on trabecular and cortical bone. The local effect of vitamin D<sub>3</sub> to enhance bone formation and achieving a better stabilization of orthodontically treated teeth have been investigated, while its effect as an accelerator for tooth eruption has not received the same attention. Although some studies focused on delayed tooth eruption as a mirror to vitamin D<sub>3</sub> deficiency<sup>(38)</sup>.

## CONCLUSIONS

In this study the systemic administration of vitamin D3 enhanced eruption rate and the acceleration was achieved from week one after vitamin D3 injection. This acceleration could be attributed to the positive effects of vitamin D3 on bone

**(Table 1) Descriptive statistics and Independent Sample T-Test of Eruption Rate of Different Groups mm/d**

	Range	Minimum	Maximum	Mean	Std. Error	Sig	T value
D3W1	.340	.730	1.070	.993	.041	0.000**	-4.326
Cw1	.145	.611	.756	.676	.021		
D3W2	.630	.540	1.170	1.023	.081	0.000**	-4.448
Cw2	.174	.573	.747	.721	.023		
D3W3	.510	.710	1.220	1.091	.070	0.000**	-5.991
Cw3	.223	.567	.765	.733	.027		

\*significant at  $P \geq 0.05$

**(Table 2) Descriptive statistics of BAP, TRACP, MMP1 and MMP3**

	Range	Minimum	Maximum	Mean	Std. Error
BAP					
Control	39.000	3.000	42.000	15.425	9.070
D3w1	160.700	3.600	164.300	102.300	34.899
D3w2	95.000	104.000	199.000	154.400	24.389
D3w3	201.100	4.2000	205.300	141.450	46.448
TRACP					
Control	48.300	3.600	51.900	31.050	10.480
D3w1	51.200	2.700	53.900	32.825	11.402
D3w2	51.100	18.200	69.300	44.050	10.556
D3w3	36.900	3.500	40.400	21.150	9.887
MMP1					
Control	4.100	3.800	7.900	5.650	.856
D3w1	4.500	3.100	7.600	5.725	.965
D3w2	5.500	5.600	11.100	7.925	1.175
D3w3	2.400	2.400	4.800	3.950	.543



MMP3					
Control	4.300	12.000	16.300	14.100	1.106
D3w1	5.400	12.300	17.700	14.250	1.190
D3w2	8.900	5.700	14.600	11.225	1.923
D3w3	6.300	7.900	14.200	11.875	1.400

(Table 3) Independent Sample T-Test between Control vitamin D Subgroups of MMP1, MMP3, BAP and TRACP Concentrations

Group	MMP1		MMP3		BAP		TRACP	
	T value	sig	T value	Sig	T value	Sig	T value	Sig
D3w1	0.058	0.956	0.092	0.929	2.409	0.085	0.115	0.913
D3w2	1.564	0.173	1.295	0.254	5.341	0.002**	0.906	0.416
D3w3	1.675	0.154	1.224	0.261	-2.663	0.07	0.687	0.518

\*significant at  $P \geq 0.05$

(Table 4) Descriptive statistics Of Blood Vessels, BTT, Osteoblasts, Osteocytes and % of Area OF New Bone Formation

Blood Vessels	Range	Minimum	Maximum	Mean	Std. Error
Control	3.00	1.00	4.00	2.500	.428
D3w1	3.00	1.00	4.00	2.666	.494
D3w2	3.00	.00	3.00	.666	.425
D3w3	2.00	.00	2.00	.678	.333
BTT					
Control	232.00	88.00	320.00	140.666	36.477
D3w1	312.00	88.00	400.00	274.666	57.704
D3w2	300.00	100.00	400.00	278.666	53.032
D3w3	20.00	380.00	400.00	396.666	3.333
Osteoblasts					
Control	9.00	3.00	12.00	7.666	1.333
D3w1	5.00	7.00	12.00	10.333	.760
D3w2	14.00	10.00	24.00	14.666	2.170
D3w3	10.00	6.00	16.00	12.000	1.712
Osteocytes					
Control	10.00	10.00	20.00	14.8333	1.72079
D3w1	23.00	7.00	30.00	17.5000	4.09675
D3w2	15.00	7.00	22.00	11.8333	2.42785
D3w3	7.00	13.00	20.00	15.8333	.98036
%ANBF					
Control	.847	.770	1.617	1.131	0.194
D3w1	1.883	7.982	9.865	8.907	0.384
D3w2	3.493	14.651	18.144	16.146	0.734
D3w3	4.659	22.573	27.232	24.533	0.988

(Table 5) Independent Sample T- Test Between Control and Vitamin D Subgroups of Blood Vessels, BTT, Osteoblasts, Osteocytes and Percentage of Area of New Bone Formation

Subgroups	Blood Vessels		BTT		Osteoblast		Osteocyte		% A.N.B.F.	
	T. value	Sig.	T. value	Sig.	T. value	Sig.	T. value	Sig.	T. value	Sig.
D3w1	0.225	0.804	1.963	0.078	1.884	0.089	0.600	0.562	21.018	0.000**
D3w2	2.803	0.019*	2.144	0.061	2.748	0.024*	1.008	0.340	0.079	0.000**
D3w3	3.379	0.008**	6.989	0.001**	.0889	0.404	0.505	0.625	23.991	0.000**

\*significant at  $P \geq 0.05$

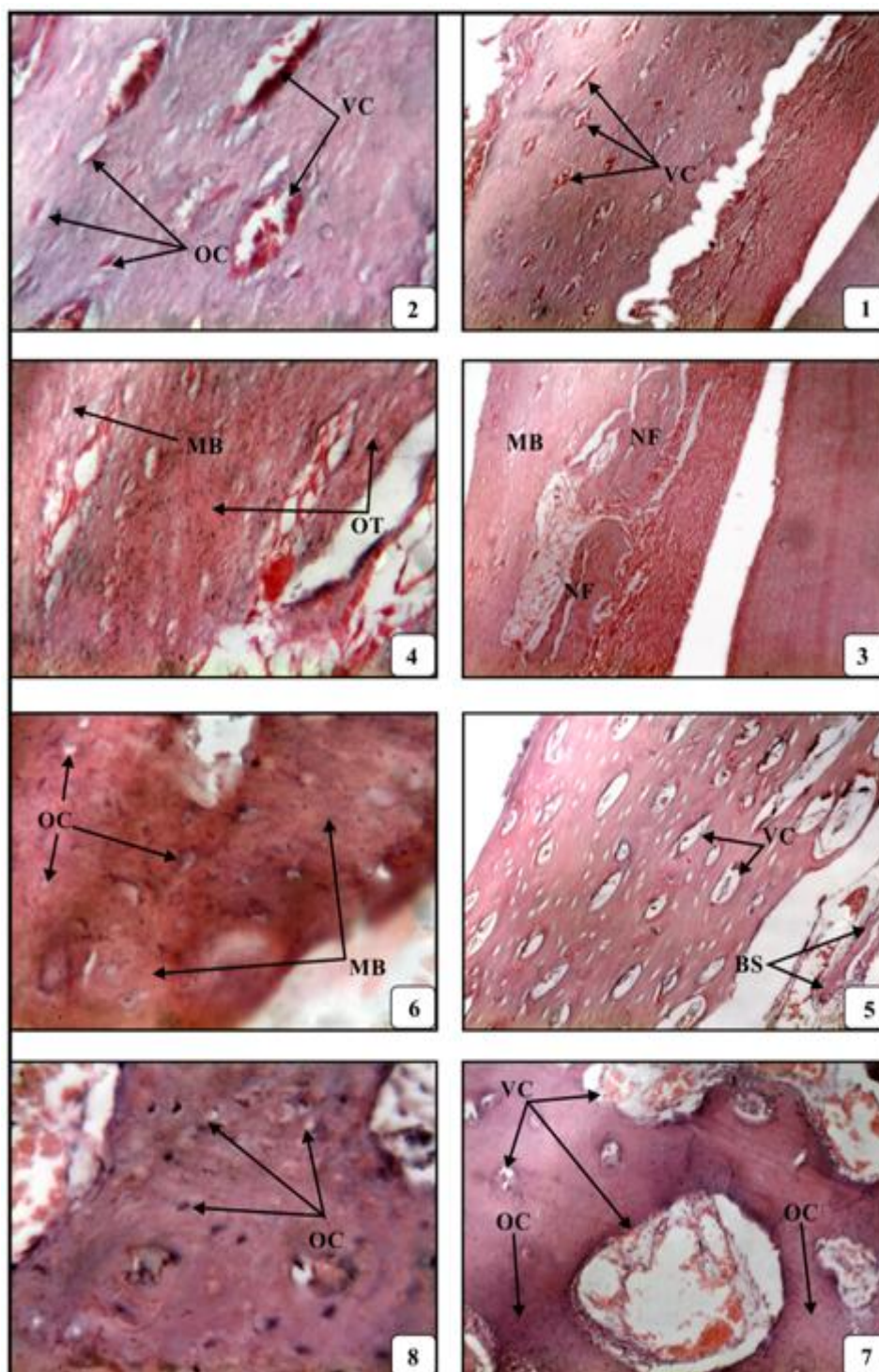


Fig. 1: Histological evaluation figures:

- 1- Control cross section of decalcified mature bone. Note the numerous vascular channels (VC). H&E, 100X.
- 2- Control photomicrograph of an area from mature bone shown in previous figure. Engorged vascular channels (VC) and osteocytes (OC) are visible. H&E, 400X.
- 3- Vitamin D week one Photomicrograph of decalcified mature bone (MB) with mass of newly formed bone (NB) attached to the mature bone of a rabbit, one week after treatment with vitamin D. H&E, 100X.
- 4- Vitamin D week one Photomicrograph of decalcified mature bone (MB) and newly formed osseous tissue (OT) attached to the mature bone. H&E, 400X.
- 5- Vitamin D week two photomicrograph of decalcified bone from a rabbit, two weeks after treatment with vitamin D. H&E, 100X.
- 6- Vitamin D week two photomicrograph of decalcified mature bone from a rabbit, two weeks after treatment with vitamin D. Numerous osteocytes (OC) could be seen. H&E, 400X.
- 7- Vitamin D week three photomicrograph of decalcified bone from a rabbit 3 weeks after treatment with vitamin D. Note the vascular channel (VC) and osteocytes (OC). H&E, 100X.
- 8- Vitamin D week three higher magnification of the previous figure. Note the numerous osteocytes (OC). H&E, 400X.

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