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# THE DUAL ROLE OF VITAMIN D IN OSTEOPOROSIS TREATMENT

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## ABSTRACT

**Background: Osteoporosis is a condition characterized by low bone mass and increased bone fragility, and increase the risk of fractures. Osteoporosis is attributed to interaction between endocrine, metabolic and mechanical factors. proinflammatory cytokines are involved in the regulation of osteoblasts and osteoclasts. There is growing evidence that vitamin $D_3$ deficiency could be a contributing factor in the development of different chronic diseases and their complications. Vitamin D plays an important role in normal calcium and bone homeostasis though stimulation of new bone formation and suppression of the production of proinflammatory cytokines. Methods: fifty rats were included in the experiment and were divided in to five groups of ten rats in each group as the following: group1: control group; group2: induced osteoporosis group; group3: induced osteoporosis received vitamin D for one month; group4: induced osteoporosis received vitamin D for two months; group 5: induced osteoporosis received vitamin D for three months. Serum samples were collected for estimation of inflammatory cytokines (IL1, IL 6, and TNF) and inflammatory cytokines (IL10 and IL13) and bone marker (RANKL, osteocalcin, and ALP) by ELISA technique. Results: vitamin D treatment suppress the inflammation and improve the immune system in addition to stimulation of new bone formation through inhibition of RANKL and stimulation of osteocalcin and ALP. Conclusion: vitamin D can be considered an effective therapeutic agent for osteoporosis.**

## INTRODUCTION

Bone is a highly complex connective tissue, composed of several types of cells including osteoblasts, which are involved in the creation and mineralization of bone tissue, osteocytes, and osteoclasts, which are involved in the reabsorption of bone tissue. Osteoporosis is characterized by a systemic impairment of bone mass and microarchitecture that results in a loss of bone strength and increased fracture risk (Rachner et al., 2011).

Bone homeostasis is maintained by the balanced function of osteoblasts and osteoclasts. Which represent a balance between bone formation and bone resorption? The OPG/RANK/RANKL system is one of the most important signaling pathways in bone metabolism.

RANKL acts as an osteoclast-activating factor secreted by activated T cells. RANKL binds to RANK receptor, which is expressed on osteoclast precursors, preosteoclasts. RANKL-RANK interaction induces osteoclast differentiation and maturation. With subsequent activation of downstream signaling pathways such as NF-kB pathway (Takayanagi et al.,2002).

OPG protects the skeleton from excessive bone resorption by acting as a soluble decoy receptor that can bind to RANKL (Min et al., 2000).

In the past, osteoporosis was considered an age-related disorder that increase with age, characterized by low bone mass and increased bone fragility and increase the risk for fractures. nowadays, it is believed that osteoporosis is a heterogeneous condition which can occur in any age of life depending on the etiology which can be caused by various endocrine, metabolic and mechanical factors like (abnormalities of parathyroid hormone and calcitonin secretion, insufficient vitamin D and calcium intake, postmenopausal hormonal condition, pregnancy, nutritional disorders, immobility and consumption of drugs such as cortisone (Yun and Lee 2004).

Furthermore, factors involved in inflammation are involved in bone physiology and remodeling, supporting that inflammation is significantly contributing to the pathology of osteoporosis (Arron and Choi 2000).

In addition to the critical role of vit D in calcium homeostasis, vit D has also plays an important role in the modulation of the immune/inflammation system via regulating the production of inflammatory cytokines and inhibiting the proliferation of proinflammatory cells, which is critical for the pathogenesis of inflammatory diseases (Yin and Agrawal 2014).

## MATERIALS AND METHODS

### Experimental animals

After obtaining an approval from Institutional Animal Care, fifty-two female 6 weeks white albino rats were cared in the animal house unit of Cairo University provided the veterinary care. Forty-two rats were induced inflammation-mediated osteoporosis (IMO) by subcutaneous injections of 16 mg/g magnesium silicate (talkum) at sites distant from the skeleton, to stimulate an acute phase response, by granulomatous reactions and accumulation of inflammatory cells (Kourkoumelis et a., 2012 and Armour 2003).

After three weeks of talkum injection, osteoporosis was confirmed by estimation of RANKL level in two randomly selected rats, Animals were randomly divided into five groups (10 animals each): ***group* 1:** a negative control; ***group* 2:** induced osteoporosis (pathological control) (Kourkoumelis et a., 2012 and Armour 2003); ***group* 3:** induced osteoporosis that received intraperotonial vitamin D treatment for one month **(Lee 1996)**; ***group*4:** induced osteoporosis that received intraperotonial vitamin D treatment for two months*;* ***group* 5:** induced osteoporosis that received intraperotonial vitamin D treatment for three months

### ELISA technique for studied marker.

Venous blood was collected from the retro-orbital vein for estimation of inflammatory cytokines (IL-1, IL-6 and TNF), the anti-inflammatory cytokines (IL-10 and IL-13) and bone marker

(RANKLE, ALP) according to kit instructions ( RayBio® Rat IL-1 beta ELISA Kit. Catalog #: ELR-IL1b RayBio® Rat IL-6 ELISA Kit. Catalog #: ELR-IL6.SIGMA ALDRICH. Rat Tumor Necrosis Factor-a (TNF-a) ELISA Kit. Catalog Number RAB0480; Quantikine® IL -10 ELISA. Catalog Number R1000; CUSABIO, Rat Interleukin 13 (IL-13) ELISA Kit Catalog Number. CSB-E07454r; cloud clone corp SEA855Ra 96 Tests. Enzyme-linked Immunosorbent Assay Kit

For Receptor Activator Of Nuclear Factor Kappa B Ligand (RANkL). Cloud clone corp SEB091Ra 96 TestsTests. Enzyme-linked Immunosorbent Assay Kit for Alkaline Phosphatase, Liver/ Bone/Kidney (ALPL); Immutopics, Inc. Rat Osteocalcin ELISA Kit. Cat.# 60-1505.

**Statistical method**

Data are expressed as mean ± SD. Significant differences were determined by using ANOVA and post hoc tests for multiple comparisons using SPSS version 22. Computer Software. Results were considered significant at $p<0.05$.

## RESULTS

**Vit D significantly lower the level of inflammatory cytokines.**

The inflammatory cytokines (IL-1, IL-6 and TNF) were significantly elevated in induced osteoporosis group, while up on vitD treatment the level of (IL-1, IL-6) significantly decreased in the first , the second and third month of treatment compared to the osteoporosis (p value<0.001)with no significant difference in their level between the two, three months treatment and control group (p value>0.05).the level of TNF is significantly decreased only after three months of vitD treatment compared to control (p value = 0.04).no significant difference in the level of (IL-1, IL-6 and TNF) between the three duration of vitD treatment (p value >0.05) (figure 1A).

**The anti-inflammatory cytokines significantly elevated on vitD treatment.**

The anti-inflammatory cytokines IL-10 and IL-13 significantly elevated on osteoporotic rats up on vitD treatment for two months and three months duration compared to both control and osteoporotic groups (p value<0.001). In addition, the level of IL-13 is significantly elevated with time (p value=0.02) (figure 1B).

**vitD treatment significantly enhance bone formation:**

The RANKL level is significantly higher in osteoporosis, one and two months of vitD treatment compared to control group (p value <0.001), while no significant difference between the treated group on vitD for three months compared to control (p value=0.8).no significant difference between osteoporosis and the groups received vitD for one month treatment (p value =0.08) while significantly decreased in groups received vitD for two and three months (0.03, <0.001).the level of RANKL is significantly decreased with continuation of vitD treatment (p value <0.001) (figure 1C).

Significant decrease in ALP and osteocalcin level in osteoporosis compared to control group (p value <0.001, 0.012) respectively; While no significant difference between all vit D treated groups and control group (p value >0.05). No significant difference in their level though out vit D treatment (p value>0.05) (figure 1D).

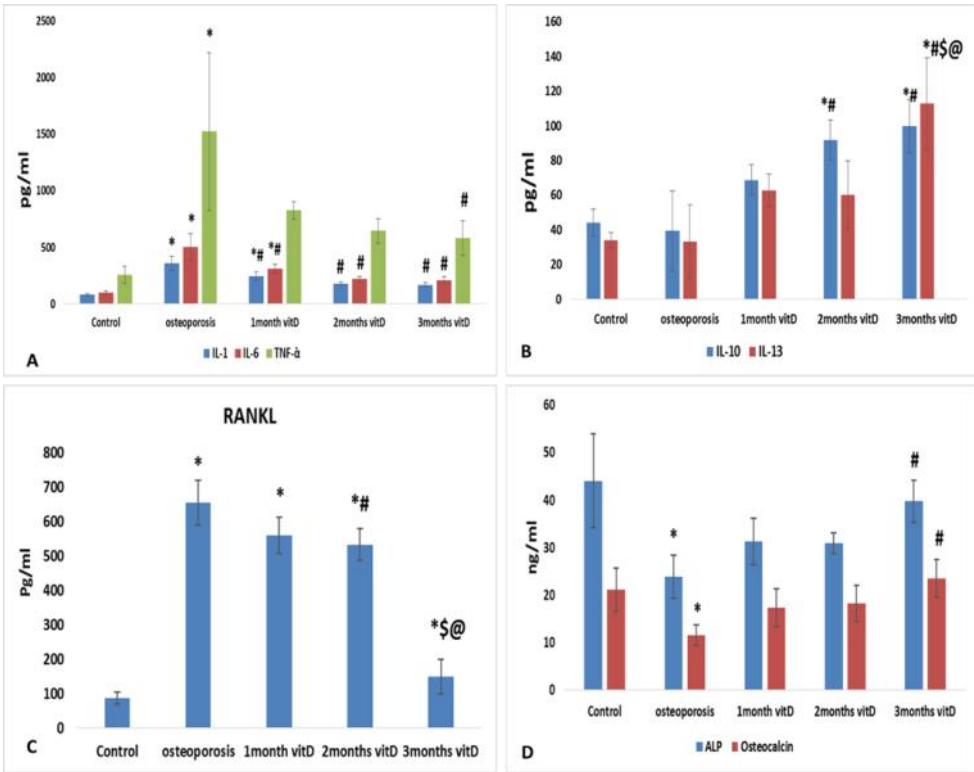


Fig (1):
Data were expressed as mean±SD. p value< is considered significant.
(*): denotes statistical significant versus control group.
(#): denotes statistical significant versus osteoporosis group.
($): denotes statistical significant vitD treated group for one month.
(@): denotes statistical significant vitD treated group for one month.

## DISCUSSION

Osteoporosis is a debilitating disease that affects millions of people worldwide. Current osteoporosis treatments are predominantly bone-resorbing drugs that are associated with several side effects. Osteoporosis is caused by an imbalance between the tightly regulated process of bone formation by osteoblasts and restoration by osteoclasts (Antebi et al., 2104).

The current study depends on induction of osteoporosis by inducing inflammation, based on previous studies which reported that the proinflammatory cytokines interleukin-1, interleukin-6, and tumor necrosis factor-alpha are important regulators of bone resorption (McLean 2009).

IL-6 promotes osteoclast differentiation and activation and involved in pathogenesis of various metabolic bone diseases. IL-1 is another potent stimulator of bone resorption that associated with the accelerated bone loss seen in idiopathic and postmenopausal osteoporosis. TNF-α is implicated in tumor-induced bone resorption and non-tumor-induced osteopenia (Ginaldi et al., 2005).

The aim of this study is to investigate the role of vit D on treatment of osteoporosis whether through alleviation of inflammation or affection of bone cells. We found significant decrease in the level of inflammatory cytokines (IL-1, IL-6 and TNFα) after vit D treatment in compare to untreated osteoporosis group; together with the elevation of the anti-inflammatory cytokines IL-10 and IL-13 up on vit D treatment which suggests that not only the anti-inflammatory role of vit D on treating osteoporosis, but also improving the immune function.

Previous studies reported that, community uses ergocalciferol showed reduction in incidence of fracture in elderly and increase in bone mineral density. Active vitamin D 1,25 (OH)2D stimulate calcium absorption of from the gut. Vitamin D deficiency causes mineralization defects, leading to osteoporosis and fractures, and muscle weakness. Thus, Vitamin D status is related to bone mineral density and bone turnover. Vitamin D supplementation may decrease bone turnover and increase bone mineral density (Lips and van Schoor 2011).

It is also agreed with another study reported that vitD Vitamin D attenuates inflammation, fatty infiltration in induced osteoarthritis model (Rai et al., 2016). More study reported that 25(OH) D is independently and inversely associated with IL-6 and positively with sIL6receptor, suggesting a potential anti-inflammatory role for vitamin D in older individuals (De Vita et al., 2014).

Although Vitamin D is known as an anti-rachitic agent preventing a failure in bone mineralization, but it is now believed that the active form of vitamin D3 (1α,25(OH)2D3) induces bone resorption. Vit D stimulate osteoclast formation in a co-culture of osteoblastic cells and hematopoietic cells. Osteoblastic cells express RANKL in response to $1\alpha,25(OH)_2D_3$. Therefore, vit D stimulate osteoclastic bone resorption. However, vit D compounds are used as therapeutic drugs for osteoporosis, because they increase bone mineral density *in vivo* due to the suppression of bone resorption. Thus, vit D has paradoxical effect both in vivo and in vitro .it can be

explained as *in vivo*, pharmacological concentrations of active vitamin D compounds in serum may alter the calcium endocrine system, which may create circumstances for the suppression of RANKL expression in osteoblasts. in addition, vit D compounds may affect the cellularity of the osteoblast lineage. As a result, the number of RANKL-positive osteoblasts decreases (Takahashi et al., 2014).

We found significant decrease in RANKL levels on the groups treated with vit D with the best results on prolongation of treatment in three months duration. this is agreed with a previous study reported that, vitamin D availability and increased VDR expression resulted in normalization of RANKL/RANK/OPG- and NF-κB-associated pathways (Labudzynskyi et al., 2018). Another study reported that 1,25 $(OH)_2D$ induces an anabolic effect in bone, through the VDR in mature osteoblasts, by increasing osteoblast activity and reducing osteoclast activity ( Goltzman 2018), more study revealed that active vit D act through the VDR to increase OPG in mature osteoblasts thus vit D plays an inhibitory role in bone resorption, through increase OPG with subsequent inhibition of RANKL (Baldock et al., 2006). Furthermore, a study on multiple myloma patients revealed that Patients with vitamin D deficiency had an increased RANKL and the RANKL/OPG ratio. With cholecalciferol supplementation in deficient patients, the average vitamin D level in deficient patients increased from 16.6 to 25.34 ng/mL while the RANKL/OPG ratio decreased (Lipe et al., 2017).

Osteocalcin is a bone matrix protein and a marker of bone formation and may be influenced by vit D. osteocalcin is produced by osteoblasts; it is often used as a marker for the bone formation process. The higher serum osteocalcin levels are correlated with increases in bone mineral density during treatment of osteoporosis with anabolic bone formation drugs. The ongoing study revealed significant increase in bone forming marker, osteocalcin and ALP with vit D treatment (Lipe et al., 2017).

The vitamin D hormone, 1,25(OH) 2D3, affects osteoblast function at multiple levels. It controls remodeling via induction of receptor activator of NF-κβ ligand, regulates phosphate homeostasis by increasing fibroblast growth factor 23, may influence energy metabolism through stimulation of osteocalcin (BGLAP), and may enhance the response of bone to mechanical loads via stimulation of mitogen-activated protein kinase signaling (Franceschi et al., 2108).

Alkaline phosphatase is a non-collagenous bone protein secreted by osteoblast and plays an important role for the mineralization of bone and represents a useful biochemical marker of bone formation. It has been reported that treatment of rat osteoblast-like cells with 1,25 (OH)2D3 promoted mineralization, which was associated with high alkaline phosphatase activity (Gerald et al., 2011).

In conclusion, vit D can improve osteoporosis through alleviation of inflammation and repression of inflammatory cytokines the key regulator of bone cells, in addition vit D stimulates osteoblast for bone formation, mineralization and decrease RANKL expression.

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## الدور المزدوج لفيتامين د في علاج هشاشة العظام

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الخلفية: ترقق العظام هو حالة مرضية تتصف بانخفاض كتلة العظام وزيادة هشاشة العظام، وزيادة كسور العظام. وتعود هشاشة العظام إلى التفاعل بين عوامل الغدد الصماء والتمثيل الغذائي و العوامل الميكانيكية. وتشارك هذه العوامل السابقة في توازن العلاقة بين osteoblasts و osteoclasts. هناك أدلة متزايدة على أن نقص فيتامين (د) يمكن أن يكون عاملاً مساهماً في تطور الأمراض المزمنة المختلفة ومضاعفاتها. يلعب فيتامين (د) دورًا مهمًا في التوازن الطبيعي للكالسيوم والعظام على الرغم من تحفيز تكوين عظام جديد ونقص إنتاج السيتوكينات المؤيدة للالتهابات. الطريقة: تم تضمين خمسين من الفئران في التجربة وتم تقسيمها إلى خمس مجموعات من عشرة فئران في كل مجموعة على النحو التالي: المجموعة الأولى: المجموعة الضابطة؛ المجموعة الثانية: مجموعة هشاشة العظام المستحثة؛ المجموعة الثالثة مجموعة :هشاشة العظام المستحثة المعالجة بفيتامين (د) لمدة شهر واحد؛ المجموعة الرابعة :هشاشة العظام المستحثة المعالجة بفيتامين (د) لمدة شهرين؛ المجموعة الخامسة: هشاشة العظام المستحث المعالجة بفيتامين (د) لمدة ثلاثة أشهر. تم جمع عينات المصل لتقييم كمية السيتوكينات الالتهابيةIL١ ، IL ٦ ، TNFα ، IL١٠، IL١٣ وعلامة هشاشة العظام RANKL ، osteocalcin، ALP بواسطة تقنية ELISA. النتائج: علاج فيتامين (د) يقلل الالتهاب ويحسن جهاز المناعة بالإضافة إلى تحفيز تكوين عظام جديد من خلال تثبيط RANKL وتحفيز osteocalcin و ALP الخلاصة: يمكن اعتبار فيتامين (د) بمثابة عامل علاجي فعال لمرض هشاشة العظام.

**٧. المصرية للعلوم الطبية ٣٩(١) يونيو ٢٠١٨: ٨٥–٩٣.**