

How to Cite:

Mahmood, H., & Abass, E. A. A. (2022). Association between periostin and bone minerals in osteoporosis and osteopenia Iraqi patients. *International Journal of Health Sciences*, 6(S4), 9633–9644. <https://doi.org/10.53730/ijhs.v6nS4.12030>

Association between periostin and bone minerals in osteoporosis and osteopenia Iraqi patients

Hajer Mahmood

Ministry of Education

Corresponding author email: hajer8chem@gmail.com

Eiman A A. Abass

Department of Chemistry, Collage of Education for Pure Sciences Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq

Abstract---Background: osteoporosis is characterized by a reduction in bone mineral density, skeletal microstructure breakdown, increased bone fragility, and fracture susceptibility. Osteopenia is the preceding step to osteoporosis because it causes a decrease in bone mass, osteoporosis reduces a person's quality of life. Periostin (encoded by Postn), its name is derived from the fact that it was first detected in periosteal osteocytes and osteoblasts. Periostin deficiency has been linked to osteoporosis and weak bones. Study objectives: The purpose of this study was to determine periostin levels in serum of Iraqi patients with osteoporosis and osteopenia, and it is also possible to consider periostin as a diagnostic factor to follow the progression of osteopenia to osteoporosis. Subjects & Methods: This study was conducted between October 2021 to March 2022 in Medical City of Baghdad /Teaching Hospital. The current study included 78 individuals (females and males) aged 40-65 years' old, 53 of them are patients and 25 as a control. The lumbar spine's bone mineral density was determined using dual energy x-ray absorptiometry (DEXA)scan to diagnose these patients. Patients divided into (27) person as osteoporosis patients and (26) as osteopenia patients. Results: The current study showed an important increase in serum POSTN of osteoporosis and osteopenia patients groups when compared with control, also, there was a significance increase in BMD, T-score and calcium levels. also, there was no significance increase in Vitamin D and Phosphorous levels. Conclusions: periostin may be used as a good biomarker for diagnosing patients with osteoporosis, and it can be used to follow the progressive of osteopenia to osteoporosis disease in these patients.

Keywords---osteoporosis, osteopenia, periostin, vitamin D, calcium, phosphorous.

Introduction

Osteoporosis (OP) is a systemic skeletal disease characterized by reduced bone mineral density (BMD), impaired bone mineralization or microarchitecture, and/or decreased bone strength, it's a symptomatic disease remains undiagnosed until manifest a fracture [1]. It is a progressive metabolic or skeletal condition that increases the risk of fractures as a result of deteriorating bone density and flaws in its microarchitecture. Young adults of the same race and sex who have bone mineral density that is less than 2.5SD below the reference range are said to have osteoporosis (t score of -2.5) [2]. The uncoupling of bone resorption and production and the imbalance between osteoclast and osteoblast activity are both factors in bone loss [3]. Osteoporosis has emerged as a serious threat to human health that ultimately results in decreased daily activity, a decline in life quality, and an increase in death [4]. Osteopenia is the preceding step to osteoporosis because it causes a decrease in bone mass, osteoporosis reduces a person's quality of life, and bone mass gradually increases throughout the growth process until the mid-thirties when it begins to decline by as much as 1% each year [5].

Periostin, also known as osteoblast-specific factor, is a protein that is produced by osteoblasts (OSF-2), is made up of POSTN gene. It's an 836-amino-acid extracellular matrix protein having a molecular weight of around 93 kDa [6]. It was first described in 1993 and initially named osteoblastic-specific factor 2 (OSF-2) [7]. It plays an important role in bone growth and fracture healing. Periostin can now be found in both serum and plasma. When it comes to bone metabolism [8]. Periostin deficiency has been linked to osteoporosis and weakened bones [9]. The regulation of collagen cross-linking and osteoblast cell adhesion by periostin has demonstrated that it is essential for maintaining bone microarchitecture and bone strength [10]. Additionally, periostin has linked to a number of clinical conditions. In bone physiology, it also plays a factor in cell adhesion. In response to mechanical loading, periostin is necessary to modify bone mass and extracellular matrix (ECM) architecture [11].

Vitamin D is a steroid hormone that is well known for its effects on calcium and mineral metabolism [12]. It is a fat-soluble vitamin and considered a group of sterols. Vitamin D₂ and D₃ have high activity, vitamin D production in the skin by exposition sunlight, also considered biologically inactive and suffered by two alternate hydroxylation in the kidney and liver to become the biologically active 1,25 dihydroxy vitamin [13]. Vitamin D promotes bone health and helps to avoid osteoporosis and fractures. Vitamin D deficiency and inadequate dietary vitamin D are frequent in the older people, and are linked to an increased higher risk of fractures [14]. Vitamin D insufficiency is linked to insufficient bone mass or insufficient bone remodeling, which can lead to bone fragility and an increased risk of fractures, Vitamin D controls the interaction of osteoblasts, osteoclasts, and osteocytes. Excess non-mineralized bone matrix, bone loss, and early bone development are histopathological characteristics of vitamin D insufficiency linked to bone loss [15].

Calcium is an essential element of the human body, it form 99% from the skeleton. Calcium homeostasis has an essential role in keeping the activity of the body, like keeping the skeleton, vascular activities, transmission of nerve impulses regulation of hormonal secretion. The calcium in dietary is generally absorbed by the small intestine, during circulation and precipitated into bones, the Ca^{++} residue is excreted by urine and feces [16]. For the human body to operate properly, calcium is an essential nutrient. This macroelement is crucial for bone development, growth, and maintenance as well as for the stability of the cellular cytoskeleton. It affects a variety of extracellular and intracellular activities [17].

Phosphorous is an important mineral for cell structure, energy transfer, signaling, and other essential functions. This element has an important role in many cellular functions forming the essential content of phospholipids, Nucleic acids, coenzymes and having an effect in many functions in the body, such as formation of hard tissue structures, such as teeth, bones, and scales [18]. Phosphorous deficiency leads to rickets, stopped growth in children, and osteoporosis in adults. Phosphorous decreasing is very uncommon in humans because many sources of it are found in food except a special states like refeeding syndrome, starvation or parenteral malnutrition is hypophosphatemia noticed in healthy persons, and disorder of the kidneys' reabsorption of phosphate. Phosphorous homeostasis is kept through absorption and excretion in the gastrointestinal tract, shifts in and out of the bone, and filtration and absorption in the kidneys [19].

Materials and Methods

Study design

This study was performed between October 2021 to March 2022 in Baghdad province. The current study included 78 individuals (females and males) aged 40-65 years' old, 53 of them are patients and 25 as a control. By using a dual energy x-ray absorptiometry, the bone mineral density (BMD) of the lumbar spine was measured in these individuals who visited a clinic at the Medical City of Baghdad Teaching Hospital (DEXA).

The individuals were classification according to T.score to three groups as :

1. (25) individuals as healthy control (C).
2. (27) individuals as osteoporosis patients (Poros).
3. (26) individuals as osteopenia patients (peni).

Samples and data collection is subject to the ethics of scientific research. Exclusion criteria were patients with thyroid diseases as well as DEXA scans of other regions aside from the lumbar spine and hip joint.

Sample collection

After detecting illness using a DEXA equipment, five milliliters of venous blood were extracted from each subject. After an overnight fast, blood samples from patients and healthy volunteers were taken. Then serum and whole blood were

stored at (-20 °C) for using later in laboratory assessments, which encompassed Vitamin D3 (Vit.D3), Calcium (Ca), Phosphorous (P) and Periostin (POSTN).

Methods

DEXA stands for Dual Energy X-ray Absorptiometry: The World Health Organization (WHO) has defined diagnostic criteria for charic osteoporosis and DXA fracture risk assessment. Osteoporosis is defined as a BMD value at the spine or hip that is more than 2.5 standard deviations below the optimum mean for healthy young persons of the same race and gender (T- score -2.5) [20].

Determination of POSTN (ng/ml) Levels in Blood Serum: The enzyme-linked immunosorbent assay (ELISA) kit was purchased from (Fine Test, China) and used for determination of the levels of POSTN. Sandwich ELISA (Human, Germany) format was employed and performed as per the manufacture 's instructions.

Determination of Vitamin D3: The Mini VIDAS 25-OH Vitamin D Total Assay design is based on a 2-step competitive immunoassay.

1. Serum or plasma 25(OH)D is separated from its protein carrier (DBP) and then mixed with a Vitamin D-specific antibody that has been alkaline-phosphatase (ALP) conjugated.
2. The vitamin D analog coated-solid phase receptor is then exposed to the unbound ALP-antibody. The substrate reagent is then added to start the fluorescence reaction after washing the solid-phase. The quantity of 25(OH)D in the sample and the number of relative fluorescence units the system detects are inversely correlated. Normal value: 30-57 ng/dl

Determination of calcium: Calcium was determined utilizing mindary BS-230 clinical chemistry analyzer full automated instrument using close system laboratory kit for this purpose, the principle of determination was based on the enzymatic reaction. Normal range (8-10 mg/dl).

Determination of Phosphorous: Phosphorous was determined utilizing mindary BS-230 clinical chemistry analyzer full automated instrument using close system laboratory kit for this purpose, the principle of determination was based on the enzymatic reaction. Normal range (3.4 to 4.5 mg/dl).

Statistical Analysis

The results were done using means $\pm$ SD; t-test was used to estimate the variances between different sets. P-values of ($p \leq 0.05$), and ($p > 0.05$), were regarded statistically a significant, and non-significant, respectively. The correlation coefficient (r) was investigated and utilized to describe the relationship between the various studied parameters. The cut off value, sensitivity and specificity were calculated by applying Receiver operative characteristics (ROC) curve by using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft office 2007.

Results

This study involved 53 osteoporosis and osteopenia patients and 25 healthy control; their ages between 40-65 years. The levels of BMD, T-score, POSTN, Vit.D3, Ca and P levels in control (C), patients with osteoporosis (Poro) and patients with osteopenia (peni) groups were summarized in Table 1. The results which expressed as (mean $\pm$ SD), showed a significance ($p \leq 0.05$) different in BMD, Tscore, POSTN and Ca levels in both (Poro) and (Peni) patient groups when comparing with control group (C).

The mean value of serum Vit.D3 and P levels in Table 1, showed no significance ($p > 0.05$) different in (Poro) and (Peni) patient groups when comparing with control group (C).

Table 1. Mean $\pm$ SD of studied parameters levels in control, osteoporosis (Poro), and osteopenia (Peni) patients groups

Groups Parameters	Control No. (25)	Poro patients No. (27)	Peni patients No. (26)
Male/Female	9/16	7/20	13/ 13
BMD (g/cm ²)	1.05 $\pm$ 0.05	0.67 $\pm$ 0.08 <i>a^S</i>	0.88 $\pm$ 0.04 <i>b^S c^S</i>
T-Score	-0.35 $\pm$ 0.4	-3.48 $\pm$ 0.7 <i>a^S</i>	-1.71 $\pm$ 0.38 <i>b^S c^S</i>
POSTN (ng/ml)	0.92 $\pm$ 0.05	2.03 $\pm$ 0.78 <i>a^S</i>	1.34 $\pm$ 0.44 <i>b^S c^S</i>
Vit.D3 (ng/ml)	21.23 $\pm$ 6.68	20.49 $\pm$ 8.17 <i>a^{NS}</i>	20.92 $\pm$ 6.78 <i>b^{NS} c^{NS}</i>
Ca (mg/dl)	9.7 $\pm$ 0.52	8.16 $\pm$ 1.6 <i>a^S</i>	8.02 $\pm$ 1.67 <i>b^S c^{NS}</i>
P (mg/dl)	3.11 $\pm$ 0.52	3.31 $\pm$ 0.44 <i>a^{NS}</i>	3.62 $\pm$ 0.97 <i>b^{NS} c^{NS}</i>

Significant (S): $p \leq 0.05$; Non-significant (NS): $p > 0.05$

a: t-test between control and Poro patients groups; b: t-test between control and Peni patients groups; and c: t-test between Poro and Peni patients groups

Correlation of POSTN with BMD, T-score, Vit.D, Ca and P

Correlation coefficients (r) and p- values between serum POSTN with BMD, T-score, Vit.D, Ca and P ratio in Control, Osteoporosis (Poro), and Osteopenia (Peni) patients groups. shown in Table2.

Table 2. Correlation between Periostin and other study parameters in Control, Osteoporosis (Poro), and Osteopenia (Peni) patients groups

Parameters \ Groups	Correlation coefficients(r)			P-value		
	Control	Poro	Peni	C	Poro	Peni
BMD (g/cm ²)	0.21	-0.41	-0.04	0.000	0.000	0.000
T-score	0.3	-0.31	0.05	0.000	0.000	0.000
Vit.D (mg/dl)	0.0019	0.203	0.03	0.000	0.000	0.000
Ca (mg/dl)	0.19	-0.503	-0.26	0.000	0.000	0.000
P (mg/dl)	-0.31	0.102	-0.09	0.000	0.000	0.000

Highly Significant: $p \leq 0.001$

There was a highly significance positive correlations between serum POSTN with BMD ($r=0.12$), T-score($r=0.3$), Vit.D($r=0.0019$), Ca($r=0.19$), and highly significance negative correlation between POSTN with P ($r = - 0.31$) in control group (C). POSTN showed a highly significance positive correlation with Vit.D ($r =0.203$) and P ($r = 0.102$), also there are a highly significance negative correlation between POSTN with BMD ($r = -0.41$), T-score ($r=-0.31$) and Ca ($r = - 0.503$) in osteoporosis group (Poro). In osteopenia group (Peni), there was a highly significance positive correlation between POSTN with T-score ($r = 0.05$) and Vit.D ($r = 0.03$), in addition to a highly negative correlation between POSTN with BMD ($r=-0.04$), Ca ($r=-0.26$) and P ($r=-0.09$).

Receiver Operating Characteristic (ROC) Curve of POSTN between osteoporosis and control groups.

A statistical model known as the Receiver Operating Characteristic (ROC) curve uses a plot to examine the relationship between sensitivity and 1-specificity in order to determine the sensitivity and specificity that are optimal for a diagnostic test. The cut-off points estimated, which correspond to many places on the depicted curve, are used to determine if test results are positive [21].

The preeminent cut-off point that is derived from the plotted ROC curve reveals the probability of disease presence which concerns with positive test "sensitivity" and the probability of disease absence which concerns with negative test "specificity" [22] ROC test for POSTN marker in osteoporosis and control groups showed perfect cut off value with 100% sensitivity and 100% specificity, that indicates considered as a good diagnostic marker. The cutoff value upper than 1.02 representatives of patient. shown in chart (1).

Table 3. Sensitivity, specificity & cut-off value of POSTN for diagnosis of osteoporosis

ROC of POSTN	Sensitivity	Specificity	Area under curve	Accuracy		Cut off value (ng/ml)
				LB	UB	
Control & osteoporosis	100%	100%	1.00	1.00	1.00	1.02

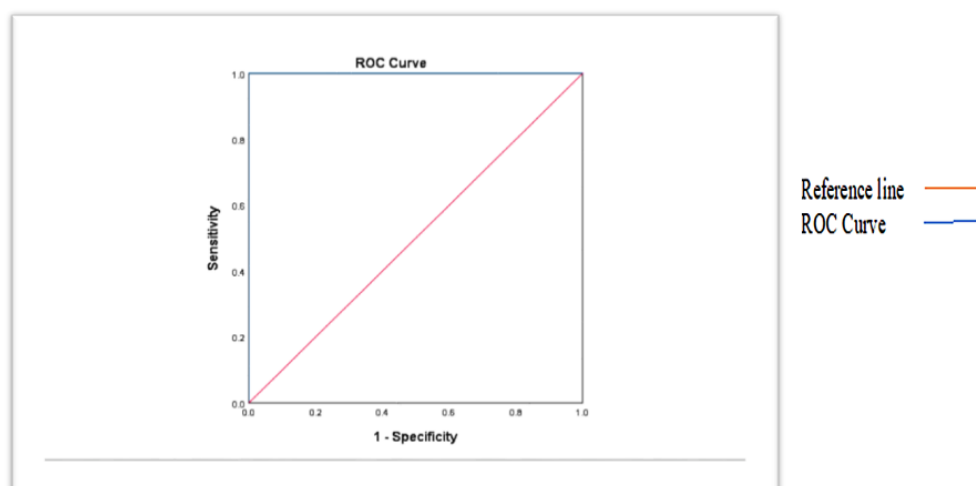


Chart (1): The Roc curve for POSTN in osteoporosis and control groups

Discussions

This study found that people with osteoporosis have a lower mean bone mineral density of the lumbar spine than people with osteopenia and healthy people. This finding is in line with other studies, such as the Almajeed and Hamdan study in Iraq and the Cranney et al. cohort study in Canada, which both found that the bone mineral density of the lumbar spine and the T-score were significantly lower in patients with osteoporosis when measured using WHO criteria. The most effective diagnostic method for detecting osteoporosis patients continues to be BMD assessment. Subsequent changes in BMD offer an unreliable predictor of treatment effectiveness, despite being useful in assisting decisions to begin osteoporosis treatment. Clinical trial analysis reveals a shaky link between rising spinal BMD and lowered risk of vertebral fracture. In most cases, the overall reduction in fracture risk is less than 25% and is not entirely attributable to increased BMD. As a result, the most clinically important therapeutic result of osteoporosis treatment is still the reduction in fracture risk [23].

T-score was measured by Double-energy X-ray absorptiometry (DEXA) scanners, this test was routinely used for osteoporosis diagnosing by measuring the BMD of the central and axial skeleton [24]. The World Health Organization's criteria for lumbar spine osteoporosis and low BMD were used in the health assessments. The definition of osteoporosis was a T-score less than or equal to 2.5 SDs below that of a young, healthy adult female reference group. When the lumbar spine T-score value was between -1 and -2.5 SDs below that of the youthful reference group, poor BMD was detected [25].

Periostin deficiency has been linked to osteoporosis and weak bones [9]. In this study, we found a significant increase in periostin levels in patients with osteoporosis. The results of a number of *in vitro* and animal experiments support our findings [26]. Periostin levels were significantly higher in individuals with osteoporosis than in healthy control group [27]. Increase in periostin expression that drives an increase in bone formation would not be sufficient to compensate

the high bone resorption. Similarly study by Kim *et al* [28], The observation that periostin levels are higher in postmenopausal women with lower BMD and osteoporotic fracture may point to the existence of compensatory mechanisms, in which periostin expression is increased to combat poor bone health.

In Rousseau *et al* [29], Periostin levels were significantly higher in both patient groups compared to the control group. This suggests that a high periostin level was linked to a higher risk of fracture. These findings matched those of Bonnet *et al.*, who discovered that periostin levels were higher in females with incidence fractures than in those without [30]. Varughese *et al.* reported elevated serum periostin levels in response to bone injury and repair. Periostin levels were also found to be higher in patients who had radiographic indications of an osteoporotic fracture [31].

In this study, there was no significant effect of vitamin D on BMD, this result was corresponded with Elisabeth *et al.* study which proven that Due to ineffective dietary calcium and phosphorus absorption, vitamin D insufficiency affects bone mineralization. Clinical signs of severe vitamin D insufficiency are osteomalacia in adults (existing bones) and rickets in children (growing bones), which are indicated by 25-hydroxyvitamin D (25(OH)D) values 25 nmol/L [12]. Zhou, P. *et al* pointed in their study in 2017 that the levels of vitamin D showed no change with osteoporosis that depended on bone mineral density [32]. and this result concurs with our study. In a previous study in 2017, Alkhenizan, A. *et al* revealed that vitamin D level dose not correlate with decreasing bone mineral density [33], Therefore, vitamin D cannot be considered a diagnostic marker for osteoporosis, especially in advanced stages if these patients are taking vitamin D treatment, but it can have a diagnostic role at the beginning of the disease, for this reason, it can be considered as a risk factor for osteoporosis.

To optimize and maintain bone density, one must consume enough calcium (Ca). A diet low in calcium is a significant risk factor for osteoporosis [34]. As bone is always being broken down and rebuilt and thus has a constant need for calcium, calcium is the main preventative measure for bone against osteoporosis. Skeletal calcium is indirectly impacted by dietary calcium consumption [35]. A decrease in the amount of calcium in food combined with factors that limit gastrointestinal absorption causes a reduction in the amount of calcium in the blood. The body attempts to make up for this shortage by taking calcium and phosphorus from the bones, which weakens them. This explains why the osteoporosis group's Ca % is lower than that of the control group [36].

Mishra *et al.* discovered that there was no significant difference in phosphorus levels in the postmenopausal group [37]. Al-Khakani, M. *et al* in 2018 showed that phosphorus levels have a significant deifferent the diagnosis of osteoporosis [32], and this was disagreed with our study. Since osteoporosis produces a decrease in aggregate mineralized bone without a decrease in the proportion of bone mineral to organic matrix, serum phosphorus was normal in both osteoporosis and osteopenia groups with and without T2DM, as well as the healthy group. As a result, there is a decrease in the overall amount of bone. The mean serum phosphorus in the patients' group was 3.31 0.44 mg/dl, whereas the control group's mean was 3.11 0.52 mg/dl, and the normal range for blood

phosphorus is 2.2–5 mg/dl, therefore both groups were within normal limits. A few studies found that serum phosphorus levels were of little value in the diagnosis of osteoporosis since the results were within the normal range [38].

Conclusions

From the current study, it was concluded that periostin may be used as a biomarker for diagnosing patients with osteoporosis because ROC test for periostin marker in osteoporosis and control groups showed perfect cut off value with 100% sensitivity and 100% specificity, that indicates it considered as a good diagnostic marker for osteoporosis patients. Also, the significant difference in the levels of periostin between osteoporosis, osteopenia patients, and control, as well as the significant inverse relationship between periostin and BMD, it can be used to follow the progressive of osteopenia to osteoporosis disease in these patients.

References

1. Alkhenizan A, Mahmoud A, Hussain A, Gabr A, Alsoghayer S, Eldali A. The relationship between 25 (OH) D levels (Vitamin D) and bone mineral density (BMD) in a Saudi population in a community-based setting. *PLoS One*. 2017 Jan 3;12(1):e0169122.
2. AlMashhadani HA, saleh KA. Electro-polymerization of poly Eugenol on Ti and Ti alloy dental implant treatment by micro arc oxidation using as Anti-corrosion and Anti-microbial. *Research Journal of Pharmacy and Technology*. 2020;13(10):4687-96.
3. AlMashhadani HA. Corrosion Protection of Pure Titanium Implant in Artificial Saliva by Electro-Polymerization of Poly Eugenol. *Egyptian Journal of Chemistry*. 2020;63(8):2-3.
4. Alpysbaev, K. S., Djuraev, A. M., & Tapilov, E. A. (2021). Reconstructive and restorative interventions at the proximal end of the thigh and pelvic bones in destructive pathological dislocation of the hip in children after hematogenous osteomyelitis. *International Journal of Health & Medical Sciences*, 4(4), 367-372. <https://doi.org/10.21744/ijhms.v4n4.1779>
5. Anderson PH. Vitamin D activity and metabolism in bone. *Current Osteoporosis Reports*. (2017);15(5):443-9.
6. Ardito F, Giuliani M, Perrone D, Troiano G, Lo Muzio L, The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy. *Int J Mol Cell Med*. (2017) Aug 1;40(2):271-80.
7. Assel F K, Munawar AA, Nada AS, Ali HA. Biomarker significance of serum cxcl8, cxcl10 and cxcl16 in breast tumors of iraqi patients. *Baghdad Sci. J*. 2020;17(1) :199-206]
8. Balbaied T, Moore E. Overview of optical and electrochemical alkaline phosphatase (ALP) biosensors: recent approaches in cells culture techniques. *BIOSENSORS*. (2019) Sep;9(3):102.
9. Beto, J.A. The role of calcium in human aging. *Clin. Nutr Res*. (2015), 4,1–8.
10. Bonnet N, Biver E, Chevalley T, Rizzoli R, Garnero P, Ferrari S. Serum levels of a cathepsin-K generated periostin fragment predict incident low-trauma fractures in postmenopausal women independently of BMD and FRAX. *JBMR* 2017; 32:2232–8.

11. Chen, Y.; Fang, W.; Wang, C.; Kao, T.; Chang, Y.; Wu, C.; et al. Body fat has stronger associations with bone mass density than body mass index in metabolically healthy obesity. *PLoS One*, 2018, 13(11): e0206812.
12. Cosman F, de Beur SJ, LeBoff MS, et al. Clinician's guide to prevention and treatment of osteoporosis. *Osteoporosis Int.* 2014; 25(10):2359- 2381
13. Elisabeth Lerchbaum, Christian Trummer, Verena Theiler-Schwetz, Martina Kollmann, Monika Wölfler, Stefan Pilz and Barbara Obermayer-Pietsch. Effects of Vitamin D Supplementation on Bone Turnover and Bone Mineral Density in Healthy Men: A Post-hoc Analysis of a Randomized Controlled Trial. *Nutrients* (2019), 11, 731.
14. El-Motelp, B.A.A., M.T. Ebrahim and H.K. Mohamed. Salvia officinalis extract and 17 β -estradiol suppresses ovariectomy induced osteoporosis in female rats. *Pak. J. Biol. Sci.* (2021);24: XX-XX
15. Fatma Gossiel, Jessica R Scott, Margaret A Paggiosi, Kim E Naylor, Eugene V McCloskey, Nicola F A Peel, Jennifer S Walsh, Richard Eastell, Effect of Teriparatide Treatment on Circulating Periostin and Its Relationship to Regulators of Bone Formation and BMD in Postmenopausal Women With Osteoporosis, *The Journal of Clinical Endocrinology & Metabolism*, Volume 103, Issue 4, April 2018, Pages 1302–1309, <https://doi.org/10.1210/jc.2017-00283>
16. Garnero P, Cremers S. Chapter 78-bone turnover markers. In: Bilezikian JP, Martin TJ, Clemens TL. et al. editors. *Principles of Bone Biology*. 4th ed. London: Academic Press; (2019). p. 1801–39.
17. Gonzalez Gonzalez L, Alonso J. Periostin: a matricellular protein with multiple functions in cancer development and progression. *Front Oncol.* (2018); 8:225.
18. Guo, Y.-M.; Cheng, J.-H.; Zhang, H.; He, J.-W.; Yue, H.; Hu, W.-W.; Gu, J.-M.; Hu, Y.-Q.; Fu, W.-Z.; Wang, C.; et al. Serum Periostin Level and Genetic Polymorphisms Are Associated with Vertebral Fracture in Chinese Postmenopausal Women. *Genes* (2022), 13, 439. <https://doi.org/10.3390/genes13030439>
19. Harvey NC, Biver E. Kaufman JM. Bauer J, Branco J, et al. The role of calcium supplementation in healthy musculoskeletal ageing. *OSTEOPOROSIS INT.* (2017) Feb;28(2):447-62.
20. Jennifer S Walsh, Fatma Gossiel, Jessica R Scott, Margaret A Paggiosi, Richard Eastell. Effect of age and gender on serum periostin: relationship to cortical measures, bone turnover and hormones. *Bone*. (2017), 99. pp. 8-13. ISSN 8756-3282.
21. Jevtic V. End stage renal disease. *European Journal of Radiology.* (2003);46(2):83-4.
22. Kanis JA, Cooper C, Rizzoli R, Reginster JY. Executive summary of European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Aging clinical and experimental research.* (2019);31(1):15- 7
23. Kim BJ, Rhee Y, Kim CH, Baek KH, Min YK, Kim DY, et al. Plasma periostin associates significantly with non-vertebral but not vertebral fractures in postmenopausal women: Clinical evidence for the different effects of periostin depending on the skeletal site. *Bone*. 2015; 81:435-41
24. Kim KH, Lee HB. Effects of circuit training interventions on bone metabolism markers and bone density of old women with osteopenia. *J Exerc Rehabil.* (2019) Apr;15(2):302.

25. Laurent Maïmoun, Fayçal Ben Bouallègue, Anthony Gelis, Safa Aouinti, Thibault Mura, Pascal Philibert, Jean-Claude Souberbielle, Marie Piketty, Patrick Garnero, Denis Mariano-Goulart, Charles Fattal. Periostin and sclerostin levels in individuals with spinal cord injury and their relationship with bone mass, bone turnover, fracture and osteoporosis status. *Bone* Volume 127, October 2019, Pages 612-619
26. Mandrekar JN. Simple statistical measures for diagnostic accuracy assessment. *J Thorac Oncol* 2010; 5:763-764.
27. Mishra S, Manju M, Toora BD, Mohan S, Venkatesh BP. Comparison of bone mineral density and serum minerals in pre and post-menopausal women. *Int J Clin Trials*. 2015 Oct;2(4):85-90.
28. Ran Li, Xiaoyi Zhu, Mengxi Zhang, Guannan Zong, Keqin Zhang. Association of Serum Periostin Level with Classical Bone Turnover Markers and Bone Mineral Density in Shanghai Chinese Postmenopausal Women with Osteoporosis. *International Journal of General Medicine* (2021):14 7639-7646, <https://doi.org/10.2147/IJGM.S335296>
29. Rousseau JC, Sornay-Rendu E, Bertholon C, Chapurlat R, Garnero P. Serum periostin is associated with fracture risk in postmenopausal women: a 7-year prospective analysis of the OFELY study. *J Clin Endocrinol Metab*. 2014; 99:2533-9
30. S. R. Wilson, C. Peters, P. Saftig, and D. J. J. o. B. C. Brömme, "Cathepsin K activity- dependent regulation of osteoclast actin ring formation and bone resorption," vol. 284, no. 4, pp. 2584-2592, 2009.
31. Small RE. Uses and limitations of bone mineral density measurements in the management of osteoporosis. *MedGenMed*. 2005 May 9;7(2):3. PMID: 16369382; PMCID: PMC1681604]
32. Soto-Davila M, Valderrama K, Inkpen SM, Hall JR, Rise ML, et al. Effects of Vitamin D2 (Ergocalciferol) and D3 (Cholecalciferol) on Atlantic Salmon (*Salmo Salar*) Primary Macrophage. Immune Response to *Aeromonas Salmonicida* subsp. *Salmonicida* Infection. *Front Immunol*. (2020) Jan 14; 10:3011.
33. Strause, L.; Saltman, P.; Smith, K.T. et al. Spinal bone loss in postmenopausal women supplemented with calcium and trace minerals. *J Nutr*, 2014; 124: 1060-1064.
34. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, 5(1), i-v. <https://doi.org/10.53730/ijhs.v5n1.2864>
35. Tabatabaei-Malazy, O., P. Salari, P. Khashayar and B. Larijan. New horizons in treatment of osteoporosis. *DARU J Pharm. Sci.* (2017);25: 1-2
36. Takeda E, Yamamoto H, Yamanaka-Okumura H, Taketani Y. Dietary phosphorus in bone health and quality of life. *Nutrition Rev*. 2012; 70:311-321.
37. Tarantino U, Iolascon G, Cianferotti L, Masi L, Marcucci G, et al. Clinical guidelines for the prevention and treatment of osteoporosis: summary statements and recommendations from the Italian Society for Orthopaedics and Traumatology. *J Orthop Traumatol*. (2017) Nov;18(1):3- 6.
38. Teresa Cobo¹, Cristina G. Viloria², Laura Solares², Tania Fontanil³, Elena González- Chamorro¹, Félix De Carlos¹, Juan Cobo¹, Santiago Cal^{3,4}, Alvaro J. Obaya^{2,4} Role of Periostin in Adhesion and Migration of Bone Remodeling

- Cells PLOS ONE | DOI: 10.1371/journal.pone.0147837 January 25, (2016);2-19.
39. Tu KN, Lie JD, Wan CKV, Cameron M, Austel AG, Nguyen JK, et al. Osteoporosis: a review of treatment options. *Pharmacy and Therapeutics*. (2018);43(2):92.
 40. Varughese R, Semprini R, Munro C, Fingleton J, Holweg C, Weatherall M, et al. Serum periostin levels following small bone fractures, long bone fractures and joint replacements: an observational study. *Allergy Asthma Clin Immunol* (2018); 14:30.
 41. Vondracek S.F, Linnebur S.A. Diagnosis and management of osteoporosis in the older senior. *Clin Interv Aging*, 2009; 4: 121-136.
 42. ZhouP, Hu J, Xip, Zhang N, Yang B, et al. Survey on the levels of 25-hydroxy Vitamin D and bone metabolic markers and evaluation of their Correlations with osteoporosis in perimenopausal woman in Xi' an region ploS on. (2017) Jul 7;12(7): e0180366.