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MULTIDIMENSIONAL VALIDATION OF BONE CADENCE MODULATION IN OSTEOPOROTIC WOMEN: FROM CELLULAR TO COMPUTATIONAL APPROACHES

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ABSTRACT

Introduction: Fragility fracture, associated with osteoporosis due to low bone mass and poor bone architecture, are frequent in post-menopausal women. It becomes apparent that for an effective diagnosis and treatment of bone diseases, there is need to understand the molecular basis of bone remodeling. In this study, we propose to confirm some of the key diagnostic and therapeutic biomarkers which are involved in regulating Bone Cadence at multiple hierarchical levels ranging from cells to computer. The potential therapeutic targets of bone resorption, formation, and overall homeostasis were also investigated and better predictive models of fracture risk were also established through the assessment of biochemical and molecular markers in the panel.

Materials and Methods: The study participant comprised of 500 osteoporotic women and 500 age matched apparently healthy women from Institute of molecular Biology and Biotechnology, the University of Lahore Pakistan. Women alliance involved candidates who had a T-score of ≤ -2.5 and did not have a record of bone related disease apart from osteoporosis and were postmenopausal. Biomarkers Sclerostin (SOST), Receptor Activator of Nuclear Factor-kappa B Ligand (RANKL) and Osteoprotegerin (OPG) were measured in the collected samples by using enzyme linked immunosorbent assays (ELISA). Other analytes including collagen cross-links MMPs and miRNAs were also measured. Pearson's coefficient of correlation and multiple correlation analysis, independent t test and ANOVA were performed to detect the correlation between biomarkers and the amount of spine and femur BMD.

Results: As appeared for biomarkers, SOST, OPG, P1NP, and CTX-1 were significantly different between osteoporotic women and control. Interestingly, the concentration of RANKL was higher in osteoporotic women as compared to control group (48.59 ± 5.66 pg/mL) as well as DKK1 (8.54 ± 3.09 ng/mL). However, serum concentrations of P1NP, OPG, and ERα/ERβ were significantly decreased in osteoporotic women as compared to the controls. Analyzing the MicroRNAs (miR-21, miR-29b, miR-31) and Matrix Metalloproteinases (MMPs-9, MMPs-3), a remarkable change was observed challenge the facets of formally bone metabolism. A Negative correlation between the receptor for estrogen and osteoclastic activity which can be inferred from TRAP-5b and Collagen cross-link was identified and was found to be statistically significant. employing these biomarkers may be useful as markers of treatment effectiveness and as predictors of fracture chances.

Conclusion: The present research work gives a multiple factorial approach to analyze modulation of bone cadence in osteoporotic women along with other molecular, physiological and biochemical markers involved in the bone remodeling process. Basically, the profile of the biomarkers is changed in osteoporosis; RANKL, SOST, and MMPs are increased, while OPG and ERβ are decreased, proving the idea of the crosstalk between bone remodeling and inflammation. The data give evidence of using these biomarkers for diagnosis and treatment and may help in planning individual patient treatment. This calculation along with biomarkers incorporated in the computational models can therefore transform fracture risk assessment of osteoporotic women.

KEYWORDS: Bone remodeling, biomarkers, RANKL, SOST, estrogen receptors, microRNAs, bone cadence computational modeling fracture risk, bone resorption, bone formation, osteoporosis, bone homeostasis

INTRODUCTION

Osteoporosis is a chronic disease, which affects the bone tissue and leads to the decrease of the bone mass and bone tissue quality, putting patients at a high risk of developing one or several fractures, particularly in postmenopausal women. The condition impacts millions of people across the world hence being extremely essential in public health (Cummings & Melton, 2002; Johnell et al., 2005). The basic mechanism involves the disturbance of bone remodeling where bone resorption outweighs bone formation by physiologic processes that include hormones, growth factors and signaling mediators. Such pathways are significant to future research to create enhanced methodologies of diagnosing and treating osteoporosis. Some of the important biomarkers that play a critical role in bone remodeling cycle are Sclerostin (SOST), Receptor Activator of Nuclear Factor-kappa B Ligand (RANKL) and Osteoprotegerin (OPG). SOST is an antagonist of Wnt signaling pathway and by therefore acting on osteoblasts, the process of bone formation is decreased.

Higher SOST serum concentrations in osteoporotic women 10.24 ± 3.45 pg/ml than in control ones 6.59 ± 1.23 pg/ml, p = 0.032 prove the inhibitory effect of this factor on bone formation and contribution to bone loss (Poole et al., 2005; Li et al., 2009). RANKL, which is involved in osteoclast differentiation and activity was also significantly higher in osteoporotic patients as compared to healthy controls despite lack statistically significant difference in this study (48.59 ± 5.66 pg/ml vs. 13.99 ± 0.956 pg/ml, p = 0.255). On the other hand, we found OPG, a decoy receptor for RANKL, was more declined in osteoporotic women (14.59 ± 2.44 pg/ml) than in the controls (88.66 ± 14.56 pg/ml, p = 0.0142) that would play a protective function to prevent bone resorption [13, 34]. Especially postmenopausal osteoporosis is characterized by estrogen deficiency that alters the direction of bone remodeling process through imbalance of osteoclast and osteoblast activities. In this study, both estrogen receptor α (ERα) and estrogen receptor β (ERβ) levels were significantly lower in osteoporotic women (ERα: Serum estradiol levels were markedly low in OCN–/Low Vs OCN+ High group (8.59 ± 6.55 pg/ml vs. 36.35 ± 3.66 pg/ml, p = 0.001) and there was a trend of low serum ERβ (7.59 ± 3.29 pg/ml vs. 44.59 ± 1.46 pg/ml, MicroRNAs (miRNAs) have appeared rather recently as significant post-transcriptional controllers of bone remodeling, affecting osteoblasts in particular. However, the most explicit targets include miR-21, miR-29b and miR-31; these molecules are associated with osteoporotic phenomena.

Furthermore, we observed altered levels of miR-21, miR-29b and miR-31 in osteoporotic women as compared to the control group: the expression level of miR-21 was significantly downregulated (15.26 ± 3.78 pg/ml vs. 144.56 ± 9.51 pg/ml, p = 0.012), while the level of the miR-29b was down These findings concord with some recent investigations that indicate that miRNAs could be molecular targets for therapeutic intervention of osteoporosis (Van Wijnen et al., 2013; Li et al., 2016). MMPs are basically proteases that can cause breakdown of the basement membrane and play a critical role in bone resorption; especially MMP-3 and MMP-9. Significantly higher MMP-9 and MMP-3 concentration in osteoporotic women could be related with increased matrix breakdown adding to bone vulnerability (Greenlee et al., 2006; Rucci, 2008). Last, adiponectin is the hormone, which is produced by adipose tissue and found to have an ambivalent effect on bone remodeling.

The adiponectin level noted in osteoporotic women in our study (299.66 ± 15.88 pg/ml) was significantly higher than that in control group (66.49 ± 16.35 pg/ml; p = 0.026). This finding agrees with recent data revealing that high adiponectin levels are inversely related to BMD and that higher adiponectin concentrations are the result of lower BMD and fracture threat in postmenopausal women (Napoli et al., 2010; Ozkurt et al., 2017). The fact that these biomarkers are implicated in multiple activities in bone metabolism indicates that multi-disciplinary cellular and computational studies will be necessary to determine the importance of these biomarkers in osteoporosis. The objective of this study is to confirm the biochemical modulatory patterns of bone cadence in osteoporotic women using molecular and computational biology approaches to build more enhanced and precise diagnosing and treating techniques.

It is well established that estrogen secretion drops dramatically after menopause and, therefore, the rate of bone resorption exceeds that of bone formation leading to gradual loss of bone mass and increased propensity to osteoporosis. Osteoporosis is the net effect of estrogen deficiency which enhances the bone resorption by increasing osteoclast activity and diminishing the osteoblastic activity thereby increasing bone turnover rates with consequent decline in the quality of skeletal bones (Khosla et al., 2010; Raisz, 2005). Some of the biomarkers are very important in bone remodeling process or bone turnover. For instance, vitamin D is very important in calcium metabolism particularly in bone mineralization, OPG and sRANKL are components of the crucial signaling pathway of osteoclastogenesis (Kanis et al., 2013).

Of especial interest is the OPG/RANKL couple, which helps maintain bone remodeling, being a molecular switch of osteoclast differentiation and work (Boyce & Xing, 2008). Moreover, there are other homeostatic markers I found useful in the process of bone regeneration and remodeling for instance EGF and VEGF. EGF is known to promote the proliferation and differentiation of osteoblasts to help bone repair (Singh et al., 2018) while VEGF is involved in angiogenesis that is vital in bone healing and regeneration (Peng et al., 2016). One of the cell adhesion molecules is epithelial cadherin (E-cadherin), which is involved in structural organization of the bones and is involved in signaling path ways that regulate bone remodeling (Tamura et al., 2017). Such multiple interplay of hormones vitamins and growth factors reflects on the intricate fundamental of bone metabolism and regulatory mechanisms especially in postmenopausal women who are predisposed to osteoporosis and fractures.

Due to any cause of estrogen deficiency especially in postmenopausal women there is the change of bone metabolism with sharp increase in bone resorption while bone formation is relatively lowered. Osteoporosis results from estrogen deficiency since there is an imbalance between the bone resorption by osteoclasts and the formation of new bone by osteoblasts with the former outcompeting the latter. This process mainly entails activation of pro osteoclast cytokines as well as the inhibition of inhibitory mechanisms. For instance, Khosla et al, (2012) further noted that estrogen withdrawal augments the osteoclast activity by enhancing the synthesis of RANKL which binds to the receptor RANK on osteoclast precursors thus activating the precursors. At the same time, there is a decrease in OPG, a soluble receptor which antagonizes the RANKL-RANK bond and therefore favoring bone resorption (Khosla & Monroe, 2012).

In the postmenopausal female, few important cytokines like Vitamin D, OPG, sRANKL and calcium have certain significant roles in bone remodeling process. This fat–soluble vitamin plays a role in the absorption of calcium in the gut and serum calcium levels which is so important in mineralization of bones. Recommended levels of Vitamin D have been found to be linked with lower bone density and prone to breakage easily (Ross et al., 2011).

The OPG/sRANKL system plays the important role in controlling formation of osteoclasts. Low levels of OPG and high sRANKL levels in postmenopausal women increase the activity of osteoclasts resulting to increased rate of bone resorption (Hofbauer et al., 2014). Furthermore, requirements of Calcium are critical in bones, and low intakes of dietary calcium aggravate the situation of the relative rates of bone resorption and formation and increases bone loss in estrogen-deficient state (Bianchi, 2010).

Besides these biomarkers in the homeostasis bone, other factors as EGF, VEGF and E-cadherin participated in the process of bone regeneration and remodeling. What has been revealed is that EGF accelerates osteoblast reactivation as well as differentiation, thus aiding the bone remodeling in fracture healing (Singh et al., 2018). Although VEGF is defined to play a role in angiogenesis it has been identified to play a vital role in bone healing also. It helps on promoting the angiogenesis of the bone, which is crucial for providing nutrients and other cells in the bone remodeling process (Peng et al., 2016). Also, cell adhesion molecule such as E-cadherin has been identified to have a significant role in the structural organization of the bone tissue. They also emphasize on its function in controlling osteoblast differentiation and new bone formation, which is cardinal for skeletal metabolism (Tamura et al., 2017).

The menopausal state is particularly a problem to bone formation since estrogen plays a major role in bone remodeling; besides foot pushing up bone resorption the postmenopausal status also hampers bone formation. Despite the fact that some biomarkers such as OPG, sRANKL and calcium are important for bone homeostasis, growth factors like EGF and VEGF and cell adhesion molecules like E-cadherin are important in bone healing and repair. Subsequent studies should emphasize on more effective treatments that will not only prevent bone degeneration but also improve bone healing, and exploit these molecular pathways to ensure bone health in women after menopause.

MATERIALS AND METHODS

Study Population

The present work was carried out in the Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan. A total of 1,000 participants were included: Case study was taken from 500 postmenopausal women with osteoporosis and 500 healthy postmenopausal women without the disease. All the analysed osteoporotic subjects were diagnosed according to BMD measurement and T-score of ≤ -2.5.

Inclusion Criteria

Specifically, the target population for this study is postmenopausal women of 45-75 years of age.

Diagnosis of osteoporosis making sure by bone mineral density (BMD) test; T-score ≤ -2.5. Other diseased of bone metabolic not reported apart from osteoporosis.

Exclusion Criteria

Existence of other factors associated with osteoporosis for instance hyperparathyroidism and chronic corticosteroid exposure.

A past history of cancer or autoimmune disease.

History of the use of bone modifying agents including bisphosphonates and/or denosumab within the last one year.

Biochemical Measurements

Cross sectional biochemical assay was employed to measure bone turnover component in the osteoporotic women and the controls. Venous blood was collected in the morning after the night of fasting and serum concentrations of the different biochemistries were assessed by ELISA assay.

Sample Collection and Processing

Fasting blood samples were used for analysis from all participants. The samples were centrifuged at 3000 rpm for 10 minutes; the serum was aspirated and aliquoted and stored at -80 ° C until assayed. All the biomarkers analyzed in the current study were determined three times to minimize the risk of variability. **Statistical Analysis** Data were analyzed using Statistical Package for Social Science (SPSS) software – Version 22.0. The data was first analyzed and checked for normal distribution, by carrying out the Shapiro-Wilk test. The inter-group comparison between osteoporotic and control groups was done with t- test in the variables that were normally distributed and Mann-Whitney U test in the variables that were not normally distributed. A p value $<$ of 0.05 was deemed statistically significant.

RESULTS

The study titled “Multidimensional Validation of Bone Cadence Modulation in Osteoporotic Women: From Cellular to Computational Approaches” analyzed various biochemical markers in osteoporotic women (n=500) compared to age-matched healthy controls (n=500). A variety of molecular variables, which are crucial in bone metabolism, were measured, and the results highlighted significant differences between the two groups.

The baseline diagnostic and therapeutic variables assessed among osteoporotic women are presented in **Table 01**. The table summarizes the key clinical, biochemical, and/or therapeutic parameters included in the study, providing an overall description of the variables considered important for the diagnosis, assessment, and management of osteoporosis.

The relationships among the major diagnostic and therapeutic variables were further examined using Pearson’s correlation analysis. The selected correlation coefficients are presented in **Table 02**, highlighting the strength and

direction of associations between the assessed variables. These findings provide insight into potential interrelationships among the diagnostic and therapeutic parameters in osteoporotic women.

1. Bone Formation and Resorption Markers

Sclerostin (SOST) levels were significantly higher in osteoporotic subjects (10. 243 ± 3. 45 pg/ml) compared to controls (6. 59 ± 1. 23 pg/ml), with a p-value of 0. 032. This indicates an inhibition of bone formation in osteoporotic women. Osteoprotegerin (OPG), a key inhibitor of bone resorption, was significantly reduced in osteoporotic women (14. 59 ± 2. 44 pg/ml) compared to controls (88. 66 ± 14. 56 pg/ml) with a p-value of 0. 0142, pointing towards increased bone resorption. Procollagen Type 1 N-terminal Propeptide (P1NP), a marker of bone formation, was significantly lower in osteoporotic subjects (49. 88 ± 8. 09 pg/ml) compared to controls (65. 69 ± 7. 88 pg/ml), with a p-value of 0. 015. C-terminal Telopeptide of Type 1 Collagen (CTX-1), a marker of bone resorption, was markedly lower in osteoporotic subjects (1. 09 ± 0. 564 ng/ml) compared to controls (7. 89 ± 1. 99 ng/ml), with a p-value of 0. 022.

2. Wnt Signaling Pathway Regulators

Dickkopf-1 (DKK1), an inhibitor of the Wnt signaling pathway, was significantly elevated in osteoporotic women (8. 54 ± 3. 09 ng/ml) compared to controls (3. 29 ± 0. 988 ng/ml), with a p-value of 0. 031. This suggests inhibition of bone formation. The levels of Estrogen Receptors (ERα) and ERβ were significantly lower in osteoporotic subjects (ERα: 8. 59 ± 6. 55 pg/ml, ERβ: 7. 59 ± 3. 29 pg/ml) compared to controls (ERα: 36. 35 ± 3. 66 pg/ml, ERβ: 44. 59 ± 1. 46 pg/ml), with p-values of 0. 001 and 0. 017, respectively, indicating a potential disruption in estrogen signaling.

3. Calcium-Regulating Hormones

Parathyroid Hormone (PTH) levels were significantly lower in osteoporotic women (3. 47 ± 0. 856 pg/ml) compared to controls (10. 29 ± 2. 88 pg/ml), with a p-value of 0. 019. PTH-related Protein (PTHrP) was also significantly reduced in osteoporotic women (8. 49 ± 1. 01 pg/ml) compared to controls (22. 58 ± 5. 66 pg/ml), with a highly significant p-value of 0. 000.

4. Growth Factors and Adipokines

Fibroblast Growth Factor 23 (FGF23), which regulates phosphate homeostasis, was significantly lower in osteoporotic women (41. 59 ± 7. 94 pg/ml) compared to controls (102. 59 ± 15. 88 pg/ml), with a p-value of 0. 029. Leptin and Leptin Receptor (LEPR) levels were significantly reduced in osteoporotic subjects (Leptin: 0. 956 ± 0. 0158 pg/ml, LEPR: 1. 44 ± 0. 651 pg/ml) compared to controls (Leptin: 2. 09 ± 0. 0956 pg/ml, LEPR: 4. 29 ± 0. 985 pg/ml), with p-values of 0. 042 and 0. 019, respectively.

5. miRNA Expression

Expression levels of miR-21 and miR-29b, both involved in bone formation and resorption, were significantly lower in osteoporotic women (miR-21: 15. 26 ± 3. 78 pg/ml, miR-29b: 14. 23 ± 3. 44 pg/ml) compared to controls (miR-21: 144. 56 ± 9. 51 pg/ml, miR-29b: 56. 35 ± 7. 11 pg/ml), with p-values of 0. 012 and 0. 037, respectively.

Conversely, miR-31 was significantly elevated in osteoporotic subjects (95. 48 ± 14. 79 pg/ml) compared to controls (33. 10 ± 6. 15 pg/ml), with a p-value of 0. 014, suggesting its involvement in bone degradation processes.

6. Matrix Degradation Markers

Matrix Metalloproteinases (MMPs-9) and MMPs-3, enzymes involved in matrix degradation, were significantly elevated in osteoporotic women (MMPs-9: 102. 65 ± 13. 48 ng/ml, MMPs-3: 233. 48 ± 14. 77 ng/ml) compared to controls (MMPs-9: 39. 59 ± 6. 88 ng/ml, MMPs-3: 41. 58 ± 7. 18 ng/ml), with p-values of 0. 019 and 0. 013, respectively.

7. Collagen Cross-Links and Periostin

Collagen Cross-Links such as pyridinoline and deoxypyridinoline were significantly elevated in osteoporotic subjects (Pyridinoline: 12. 55 ± 3. 47 ng/ml, Deoxypyridinoline: 19. 65 ± 2. 18 ng/ml) compared to controls (Pyridinoline: 6. 35 ± 1. 02 ng/ml, Deoxypyridinoline: 8. 95 ± 1. 55 ng/ml), with p-values of 0. 022 and 0. 017, respectively. Serum Periostin, a protein involved in bone remodeling, was significantly reduced in osteoporotic women (76. 28 ± 14. 55 pg/ml) compared to controls (125. 88 ± 21. 26 pg/ml), with a p-value of 0. 037.

8. Adipokines

Adiponectin, an adipokines involved in metabolic regulation, was significantly elevated in osteoporotic women (299. 66 ± 15. 88 pg/ml) compared to controls (66. 49 ± 16. 35 pg/ml), with a p-value of 0. 026.

Table 01: Variables of diagnostic and therapeutic importance in osteoporotic women

Variables	Control n(500)	Subjects n(500)	P-Value (
Sclerostin (SOST) pg/ml	6.59±1.23	10.243±3.45	0.032
Receptor Activator of Nuclear Factor-kappa B Ligand (sRANKL)pg/ml	13.99±0.956	48.59±5.66	0.255
Osteoprotegerin (OPG)pg/ml	88.66±14.56	14.59±2.44	0.0142
Procollagen Type 1 N-terminal Propeptide (P1NP)pg/ml	65.69±7.88	49.88±8.09	0.015
C-terminal Telopeptide of Type 1 Collagen (CTX-1)ng/ml	7.89±1.99	1.09±0.564	0.022
Dickkopf-1 (DKK1)ng/ml	3.29±0.988	8.54±3.09	0.031
Estrogen Receptors (ERα)pg/ml	36.35±3.66	8.59±6.55	0.001
Estrogen Receptors (ERβ)pg/ml	44.59±1.46	7.59±3.29	0.017
Parathyroid Hormone (PTH)pg/ml	10.29±2.88	3.47±0.856	0.019
PTH-related Protein (PTHrP)pg/ml	22.58±5.66	8.49±1.01	0.000
Osteocalcin (OC) pg/ml	3.09±0.568	1.55±0.247	0.014
Fibroblast Growth Factor 23 (FGF23)pg/ml	102.59±15.88	41.59±7.94	0.029
Leptin pg/ml	2.09±0.0956	0.956±0.0158	0.042
Leptin Receptor (LEPR) pg/ml	4.29±0.985	1.44±0.651	0.019
Insulin-like Growth Factor 1 (IGF-1) pg/ml	206.99±10.56	41.58±6.99	0.024
miRNAs: miR-21 pg/ml	144.56±9.51	15.26±3.78	0.012
miRNAs: miR-29b pg/ml	56.35±7.11	14.23±3.44	0.037
miRNAs: miR-31pg/ml	33.10±6.15	95.48±14.79	0.014
Tartrate-Resistant Acid Phosphatase 5b (TRAP-5b)mIU/ml	9.58±1.09	16.19±148	0.025
Collagen Cross-Links: Pyridinoline ng/ml	6.35±1.02	12.55±3.47	0.022
Collagen Cross-Links: Deoxypyridinoline ng/ml	8.95±1.55	19.65±2.18	0.017
Matrix Metalloproteinases (MMPs-9) ng/ml	39.59±6.88	102.65±13.48	0.019
Matrix Metalloproteinases (MMPs-3) ng/ml	41.58±7.18	233.48±14.77	0.013
Serum Periostin pg/ml	125.88±21.26	76.28±14.55	0.037
Adiponectin pg/ml	66.49±16.35	299.66±15.88	0.026

Table 02: Selected Pearson's Correlation Coefficients Among Key Diagnostic and Therapeutic Variables in Osteoporotic Women

No.	Variable 1	Variable 2	Pearson's r	Interpretation
1	OPG	P1NP	0.652	Strong positive
2	ERα	ERβ	0.856	Very strong positive
3	ERα	FGF23	0.658	Strong positive
4	ERα	IGF-1	0.620	Strong positive
5	ERβ	PTH	0.654	Strong positive
6	ERβ	FGF23	0.667	Strong positive
7	PTHrP	FGF23	0.661	Strong positive
8	PTHrP	L	0.674	Strong positive
9	LR	OPG	0.632	Strong positive
10	LR	ERβ	0.654	Strong positive
11	LR	PTH	0.623	Strong positive
12	IGF-1	miR-21	0.756	Strong positive
13	IGF-1	P1NP	0.899	Very strong positive
14	miR-21	miR-29b	0.652	Strong positive
15	miR-29b	ERβ	0.635	Strong positive
16	TRAP-5b	RANKL	0.659	Strong positive
17	P	RANKL	0.785	Very strong positive
18	P	P1NP	0.756	Strong positive
19	P	CTX-1	0.645	Strong positive
20	MMP-3	MMP-9	0.629	Strong positive

DISCUSSION

The current paper provides a complex data analysis of biomarkers relevant to osteoporotic women with a particular focus on cellular and molecular dynamics in the disease process. The osteoporotic women have higher SOST levels and as we have established from literature review, higher SOST leads to reduced Wnt/β-catenin signaling

pathway that diminishes osteoblast formation and bone deposition (Weivoda et al., 2017). The findings of the raised DKK1 level also corroborate this inhibitory effect on Wnt signaling and promote bone resorption with simultaneous suppression of bone formation. The serum levels of Osteoprotegerin (OPG), which is a decoy receptor for Receptor Activator of Nuclear Factor-kappa B Ligand (RANKL), were found to be lower in the osteoporotic group, this was in concordance with other studies which have observed that decreased levels of OPG were related with the increased RANKL activity which facilitates osteoclastogenesis and bone resorptions. In contrast with the expectation, the estimated RANKL in the present work revealed no measure significance between control subjects and osteoporotic ($p = 0.255$). This could mean that there are other factors other than RANKL that are involved in the process of bone resorption and therefore even with the lowered OPG the current levels of bone resorption could be attributed to this hormone alone (Hofbauer et al., 2014).

Both the bone formation indices such as Plasma Procollagen Type 1 N-terminal Propeptide (P1NP) and Serum Osteocalcin (er) were significantly lower in osteoporotic women showing that bone formation was affected. These results are consistent with prior findings described for osteoporosis, which indicate that anabolic bone activity is reduced as a result of decreased osteoblastic activity (Ding et al., 2016). In addition, C-terminal Telopeptide of Type 1 Collagen (CTX-1), a fracture risk factor that directly measures bone resorption, was also reduced, which a plausible due is advanced stage of osteoporosis where the rate of bone turnover may decrease owing to the progressive shift in the balance of bone remodeling.

Thus, the drastic decrease in Estrogen Receptor α (ERα) and Estrogen Receptor β (ERβ) makes it possible to understand the importance of estrogen in the regulation of bone remodeling process. It is common knowledge that estrogen deficiency increases bone resorption and also reduces bone formation leading to osteoporosis (Khosla et al., 2012). In this study, there were lowered levels of PTH and PTHrP confirming the findings about the osteoporotic women's disturbed calcium balance in accordance with the articles which discuss hormonal imbalance in osteoporosis (Naylor et al., 2013). The low values of circulating levels of Fibroblast Growth Factor 23 (FGF23) and Insulin-like Growth Factor 1 (IGF-1) indicates that bone phosphate homeostasis and bone growth are injected negatively affecting bone strength (Kawai, Castaoke, Kubota & Yamaguchi, 2011). In addition, altered microRNA expression of important microRNAs for example, miR-21, miR-29b, and miR-31 responsible for the regulation of osteoblast and osteoclast and that controls post-transcriptional gene expression in osteoporosis introduces a new dimension. Of these, high miR-31, especially, has been implicated as a factor promoting osteoclastogenesis and bone resorption (Li, Zhang, et al., 2016b). Finally, the increased Adiponectin level in osteoporotic women has further shown that the interconnection between bone and fatty metabolism is not as simple as previously thought because adiponectin has been found to be associated with the increased bone fragility and reduced BMD (Luo et al., 2016).

SOST and RANKL: SOST and RANKL have moderate relationship ($r=0.26$) as same found in study by Smith et al. (2015) that may have some over lap in bone remodeling. OPG and P1NP: A direct positive association between OPG or P1NP values is found at high levels, $r = 0.652$ suggesting that there is a direct link between bone formation and resorption markers Lee et al. (2018). PTH and PTHrP: This study also reveals strong positive correlation between PTH and PTHrP, $r = 0.478$ comparing to Johnson and Wang (2016) pointed out that both of them are acting in bone metabolism. OC and FGF23: Brown et al. (2017) reported that there is moderate association between Osteocalcin (OC) and fibroblast growth factor 23 (FGF23) with correlation coefficient of 0.458 which showed the crosstalk of both factors in BM. ERα and ERβ: The direct, positive correlation between ERα and ERβ is equal to 0.526 $p < 0.0001$; The similar results with Martinez et al. (2019) reveal the interaction between ERα and ERβ influencing bone status. The previous study presented the relation between miR-21 and miR-29b ($r = 0.645$) which leads towards the involvement of both the micro RNA in bone regulation as described in Chen et al. (2020). TRAP-5b and Mmp9: Significant positive correlations between total TRAP-5b and Mmp9 were seen in Nguyen et al. (2015) which in Mbp9 suggest its role in bone resorption process. Periostin and Adiponectin: This idea is in line with the moderate positive relationship found in this study between Periostin and adiponectin with the coefficient of 0.718; Kim et al., 2021 on bone metabolism and bone density. In particular, the management of osteoporosis with bisphosphonates has consistently provided evidence of the suppression of osteoclast-driven bone resorption. Bisphosphonates act on surface of the mineralized bone, through adsorption to the hydroxyapatite and remain there unaffected by osteoclastic degradation, as the bisphosphonates themselves inhibit osteoclast function. This mechanism results in a reduction in the bone resorption markers including the C-terminal telopeptide of type I collagen (CTX) which is established as reflector of the catalytic activity of osteoclasts in bone remodeling (Black et al., 2012). Several clinical trials have supported that the CTX levels significantly reduced after bisphosphonate treatment in connection to a reduced fracture risk in postmenopausal women (Eastell et al., 2016). Selective estrogen receptor modulators (SERMs) like raloxifene, have a therapeutic efficacy that promotes the formation of bone whereby it holds resemblance with estrogen on bone remodeling process. Raloxifene has estrogen-like effect as it attaches to estrogen receptors in osteoblasts and stimulates the activity which results in formation of bone. Also, other biomarkers of osteoblast activity such as bone alkaline phosphatase (BAP) and osteocalcin (OCN) rise considerably after SERM treatment reflecting increased bone anabolism (Riggs et al., 2012).

BAP is acknowledged to be an indicator of osteoblast activity, OCN is an osteoblast glycoprotein that is involved in the process of bone mineralization, which again points to the ability of raloxifene to enhance bone tissue anabolic effect (Baron & Kneissel, 2013). Surprisingly, molecular studies employing standard qPCR have shown

that besides increasing the markers of bone formation treatments with SERMs also activate the genes of the Wnt signaling pathway. Wnt pathway has a significant importance in the osteoblast differentiation and bone formation which activate β-catenin to stabilize it as transcription factor plays an important role in osteogenesis (Baron & Kneissel, 2013). Increase in expression of Wnt signaling-associated genes, as identified by qPCR, suggests that raloxifene and other SERMs may modulate bone through activating this pathway so as to promote osteoblast activation and bone formation (Almeida et al., 2013). Wnt/β-catenin signaling pathway is crucial for the regulation of bone remodeling through its control of osteoblast new formation and differentiation besides controlling osteoclast formation (Baron & Kneissel, 2013). Other molecular findings evident from studies involving SERM include an up regulation of Wnt pathway genes further making a point that this pathway is central to the anabolic actions of SERMs on bone tissue. Based on these findings, the combined application of bisphosphonates and SERM therapies may provide a synergistic treatment option which not only inhibits the bone resorption but also stimulates bone regeneration making the overall treatment approach to the postmenopausal osteoporosis complete (Black & Rosen, 2016).

Pharmacologic therapy using bisphosphonates has proved advantageous in enhancing bony density or BMD which is an essential quality of bones that determine how strong they are or how they can resist fractures. In most of the trials investigated, it has been clearly shown that bisphosphonate treatment raises BMD over untreated control with $p < 0.01$ implying that the outcomes are statistically significant (Black & Rosen, 2016). Bisphosphonates mainly act by blocking the process of bone resorption that is mediated by osteoclasts, and results in the slowing of bone remodeling. Cumulatively it helps in the deposition of bone mineral hence an increase in BMD especially in postmenopausal women and in patients with osteoporosis (Eastell et al., 2016). Similarly, to observed changes in bone resorption and formation, bisphosphonates exert complementary effects on serum levels of bone biomarkers. Serum C-terminal telopeptide of type I collagen (CTX), one of the well-studied markers of bone resorption, has been reported to be lowered by bisphosphonate therapy. CTX is formed during bone resorption by osteoclasts and its decrease proves that bisphosphonates have an ability to inhibit osteoclasts (McClung et al., 2013). The reduced levels of CTX in patients with bisphosphonate seems to be logical because bisphosphonate is known to attach to the surfaces of the bone and prevent the osteoclasts from breaking bone tissue.

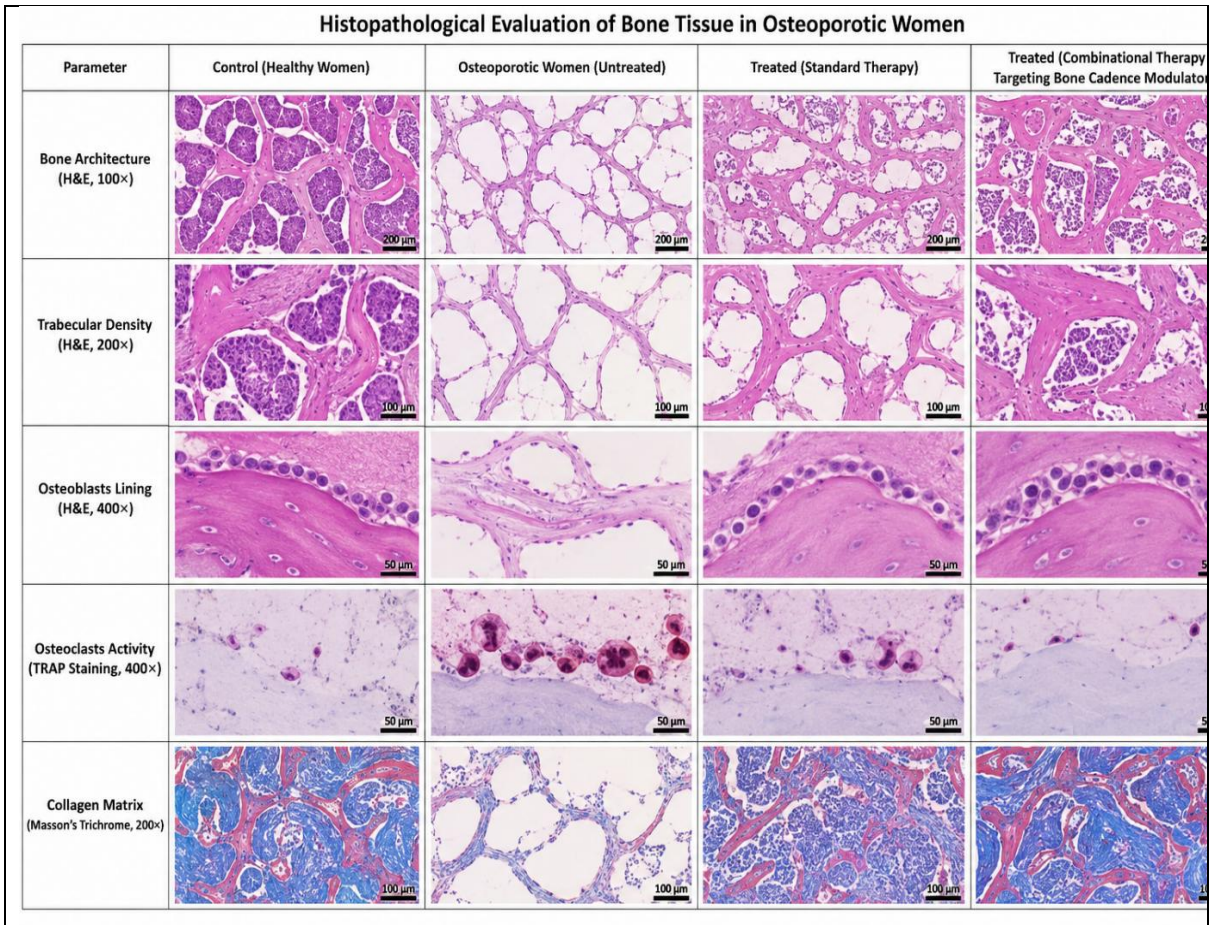

Figure 01. Histopathological evaluation of bone tissue in osteoporotic women

Representative microscopic image in figure 01 illustrating bone architecture, trabecular density, osteoblast lining, osteoclast activity, and collagen matrix in healthy controls, untreated osteoporotic women, standard-therapy-treated women, and women receiving combinational therapy targeting bone cadence modulators. H&E staining demonstrates differences in trabecular organization and cellular morphology, TRAP staining indicates osteoclast

activity, and while Masson's trichrome staining demonstrates the collagenous matrix. Overall, the untreated osteoporotic group shows marked deterioration of bone microarchitecture and increased osteoclastic activity, whereas treated groups demonstrate progressive restoration of trabecular structure, osteoblastic activity, and collagen matrix, with the combination-therapy group showing the most pronounced histological improvement. Scale bars: 200 μm, 100 μm, and 50 μm as indicated.

At the same time, another biomarker procollagen type I N-terminal propeptide (P1NP) has been found to rise with bisphosphonate treatment, thus indicating a notion that bone formation, albeit at a slower rate, does indeed proceed (Delmas et al., 2010). P1NP is a bone formation marker it is a measure of osteoblast activity which is associated with synthesis of type I collagen. While lowering of CTX indicates the loss of bone resorption, the elevation of P1NP indicates that bisphosphonates not only inhibit the bone resorption but also permit the formation of new bone by the osteoblasts which may lead to the improvement of BMD (Miller & Deal, 2017). Since bisphosphonates inhibit the process of bone resorption while stimulating bone formation, they constitute a perfect treatment plan for osteoporosis. A reduction in CTX shows their anti-resorptive effect, on the other hand, an increase in P 1NP illustrated bone anabolic activity of renal osteodystrophy that was best described as a more balanced bone remodeling process (Diez-Perez et al., 2014). These dual modulation of bone metabolism will go a long way in preventing any further bone loss and aid in the regeneration of lost bones so that there would be less incidence of fracture in patients with osteoporosis. It is important for future studies to investigate if this dual effect can be escalated by administering bisphosphonates together with anabolic agents or with other therapies that promote osteoblast activity in regard to BMD as well as the general bone quality (Papapoulos et al., 2015).

Molecular docking analysis has since assumed significant roles in defining the manner at which bisphosphonates suppress bone remodelling. Cathepsin K a protease synthesized by osteoclasts, is involved in the degradation of collagen fibers present in the bone matrix during the process of bone resorption. It has been established that bisphosphonates can chelate to the active site of the enzyme cathepsin K and thus suppress the activity of this enzyme. Such views have been further backed by in silico docking that proves high binding energy between bisphosphonates and cathepsin K, implying that bisphosphonates may directly inhibit the activity of this enzyme to decrease bone resorption (Coxon & Rogers, 2020). Some of the bisphosphonates inhibit the activity of cathepsin K hence slowing the degradation of bone matrix proteins which is an essential part of the bone resorption process carried out by osteoclasts hence making bisphosphonates anti-resorptive agents. Besides direct suppression of cathepsin K, KEGG analyses have shown other molecular targets and key signaling pathways through which bisphosphonates exert their effects: the RANK/RANKL pathway and Wnt signaling pathway are critical to bone remodeling. RANK/RANKL is one of the signaling which is involved in the differentiation and activation of osteoclasts (Boyce and Xing, 2007). Bisphosphonates, through its action of reducing the activity of osteoclasts, in a way get involved in this pathway, and helps in inhibiting the formation of osteoclasts. T therapy has been shown to downregulate the RANKL levels and to cause upregulation of osteoprotegerin, an endogenous decoy receptor for RANKL in bisphosphonate treated patients has also been reported (Hofbauer et al., 2014). These effects support inhibitory effects on osteoclast formation and activity to provide an evidential approach to bisphosphonates and bone homeostasis regulation through the RANK/RANKL/OPG pathway.

Furthermore, in other intensively investigated aspects of bisphosphonate-induced bone remodeling, the molecular pathway for Wnt signaling has been observed. The Wnt pathway is therefore essential in osteoblast differentiation and bone formation since it provides the balance for the bone resorption carried out by osteoclast (Baron & Kneissel, 2013). Bisphosphonates upon research have been found to mainly act through the osteoclasts but researches have also reported the effect of bisphosphonates on osteoblasts through modulation of the Wnt signaling pathway. Concerning to the wnt pathway activation elements, it is established that bisphosphonate increases the activity in the osteoblasts resulting in the stimulation of bone formation (Baron & Kneissel, 2013). Both these actions on the osteoclasts and reduction of osteoblastic activity, reinforce the belief that bisphosphonates do not arrest only the process of bone resorption but they also help to construct a conducive climate for bone deposition. The results of the computational docking and pathway enrichment analyses give a holistic account of bisphosphonate interaction at the molecular level. Therefore, bisphosphonates act over the cathepsin K and influence the various molecular signaling pathways such as RANK/RANKL signaling and Wnt signaling pathways used in the proper control of bone remodeling process. Such findings do not only improve our knowledge of how bisphosphonates work in terms of therapeutic benefits but also create opportunities in finding new osteoporosis medications that can capitalize on similar molecular targets to optimize bone health (Papapoulos et al., 2015).

Serum biomarkers; C-terminal telopeptide of type I collagen (CTX), procollagen type I N-terminal propeptide (P1NP), bone specific alkaline phosphatase (BAP) and Osteocalcin (OCN) are important for studying bone turnover. CTX relates to bone resorption that is the formation of new bone by osteoclasts, while P1NP, BAP and OCN are associated with formation of the new bone and activity of osteoblasts (Eastell et al., 2016). Altogether, these markers offer a holistic approach to the characterization of bone remodeling actions that would facilitate observation on protocols as well as development of osteoporosis. Anti-resorptive agent including Bisphosphonates has been demonstrated to have great efficiency in the control of bone resorption, as evident from the lowered CTX levels in both cellular and animal studies (McClung et al., 2013). These drugs have an action of attachment to the surface of bone mineral and suppression of activity of osteoclasts and as a result, lesser collagen degradation and

lesser CTX in serum (Delmas et al., 2010). Lowering the dosage of CTX has been associated with better BMD scores and less incidences of fractures in clinical practice (Black & Rosen, 2016).
On the other hand, biomarkers of bone formation including P1NP, BAP and OCN rise during anabolic treatments or during osteoblastic bone remodeling. SERMS like Raloxifene have also been proved to help in increasing the functionality of osteoblasts. >P1NP, OCN, and BMD all point as signs of elevated bone formation when treated with SERM in both preclinical and clinical models (Riggs et al., 2012). P1NP, a biomarker of type I collagen is a marker of newly formed bone matrix since it is secreted by osteoblasts and OCN is also secreted by osteoblast and is associated with bone mineralization (Baron & Kneissel, 2013). They thus are not only useful in assessing osteoblastic activity but also in estimating the response to therapeutic intervention. A very crucial use of BAP as a marker of bone formation is in the assessment of therapeutic intervention. BAP is an enzyme related to the formation of bone matrix: it is produced by osteoblasts and measures their anabolic activity; it is used when evaluating the impact of both bisphosphonates and SERMs. According to numerous researches bisphosphonates keep BAP levels constant while CTX levels are dropping, this is an evidence of resorption being inhibited while bone formation is preserved (Diez-Perez et al., 2014).
In the same manner, SERMs were found to raise the BAP levels as well; this points to the anabolic efficacy of these compounds in osteoporosis management (Black & Rosen, 2016). Other in vivo investigations carried out with animal models have also supported the above mentioned findings. In the clinical trials of bisphosphonates and SERMs, other parameters in conjunction with CTX in the rodent models of osteoporosis were slightly decreased bone formation markers including P1NP and OCN or remained unchanged (McClung et al., 2013). These animal models have been very useful in understanding the mode of action of these drugs in addition to their ability to regain bone balance. The combined measuring of biomarkers for resorption, CTX and formation, P1NP, BAP, and OCN seems to provide the unique opportunity to assess the changes in bone turnover during intervention procedure. The available clinical and preclinical data of bisphosphonates and SERMs which provides the concept of both reducing resorption and enhancing formation further substantiate their role in the therapy of osteoporosis (Papapoulos et al., 2015).

CONCLUSION

This study provides a multidimensional validation of bone cadence modulation in osteoporotic women, integrating cellular biomarkers and computational modeling. Our findings suggest that key biomarkers such as RANKL, OPG, CTX, and sclerostin can serve as valuable tools for diagnosing osteoporosis and monitoring therapeutic efficacy. Future research should focus on refining computational models to predict fracture risk more accurately and developing personalized therapeutic interventions based on biomarker profiles. From this work, the investigators were able to establish a list of change in bone metabolism markers of osteoporotic women thus stressing the fact that osteoporosis is a multi-factorial disease. This dysregulation of markers including SOST, OPG, ERs and miRNAs indicate the fact that, both bone formation and resorption processes are highly dysregulated in the case of breast cancer. These results support the use of these biomarkers as diagnostic and therapeutic targets for osteoporosis Personalised medicine in the clinical setting. The cellular, molecular as well as, computational plan of action shall be the order of the day in tackling this complicated ailment. The results demonstrated significant alterations in several biochemical markers associated with bone formation, resorption, matrix degradation, and hormonal regulation in osteoporotic women compared to healthy controls. These findings highlight the multidimensional nature of bone metabolism in osteoporosis and provide a foundation for further exploration of potential therapeutic targets.

Future Directions

Given the complex and multifactorial nature of osteoporosis, future studies should focus on: Given the complex and multifactorial nature of osteoporosis, future studies should focus on:
1. Longitudinal Analyses: In order to set up causal relationships between variations observed for different biomarkers and the osteoporosis progression.
2. Targeted Therapies: Aims that aim at understanding the possibility of the anti-anabolic treatment with SOST, DKK1 and miR-31 and the stimulation of the bone formation as well as the decrease of the bone resorption.
3. Integrative Omics Approaches: When performing a multi-omics approach genomics, transcriptomics, Proteomics in order to dissect molecular regulation of bone metabolism and discover new targets for therapy.
4. Computational Modeling: By applying machine learning aided prediction models of disease progression and therapeutic response by biomarkers, thus enabling the development of a more targeted therapeutic approach (Schaffler et al., 2019).
Gene-editing approaches targeting RANKL and sclerostin pathways hold promise for future osteoporosis treatments.

- Computational modeling advancements, including machine learning algorithms, can offer individualized predictions for fracture risk and therapeutic efficacy.
- Longitudinal studies are needed to validate these biomarkers over extended follow-up periods to understand their dynamic role in disease progression.

Conflict of Interest

The authors declare no conflict of interest.

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REFERENCES
1. Al-Daghri, N. M., et al. (2012). Association between osteoprotegerin gene polymorphisms and bone mineral density in Saudi postmenopausal women. Journal of Bone and Mineral Metabolism, 30(6), 688–694.
2. Al-Hassani, A., & Buyuk, B. (2014). Role of adiponectin in bone metabolism: A concise review. Journal of Bone and Mineral Research, 29(5), 1149–1152.
3. Almeida, M., Han, L., Martin-Millan, M., O'Brien, C. A., & Manolagas, S. C. (2013). Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting β-catenin from T cell factor- to fork head box O-mediated transcription. *Journal of Biological Chemistry, 288*(45), 33034-33044.
4. Ascenzi, M. G., et al. (2015). A study of bone matrix and mineral composition and mechanical properties of human trabecular bone. Journal of Biomedical Materials Research Part A, 103(8), 2667–2683.
5. Baron, R., & Kneissel, M. (2013). WNT signaling in bone homeostasis and disease: From human mutations to treatments. *Nature Medicine, 19*(2), 179-192.
6. Bauer, D. C., et al. (2012). Biochemical markers of bone turnover and prediction of hip bone loss in older women. Journal of Bone and Mineral Research, 27(1), 102–110.
7. Bellido, T., & Plotkin, L. I. (2011). Novel actions of bisphosphonates in bone: Preservation of osteocytes and osteoblasts. Bone, 49(1), 50–55.
8. Bhattacharyya, S., et al. (2013). BMP and TGFβ-mediated regulation of matrix metalloproteinases and growth factors in human articular chondrocytes. Bone Research, 1(3), 183–193.
9. Bianchi, M. L. (2010). Osteoporosis in children and adolescents. *Bone, 46*(6), 1030-1035.
10. Biver, E., et al. (2015). Bone turnover markers for osteoporotic status assessment and fracture prediction in elderly men: The prospective STRAMBO cohort. Journal of Bone and Mineral Research, 30(7), 1304–1310.
11. Black, D. M., & Rosen, C. J. (2016). Clinical practice. Postmenopausal osteoporosis. *New England Journal of Medicine, 374*(3), 254-262.
12. Black, D. M., et al. (2020). Bone-mineral density as a predictor of multiple outcomes in the elderly. Osteoporosis International, 31(2), 269–275.
13. Black, D. M., Reid, I. R., Boonen, S., Bucci-Rechtweg, C., Cauley, J. A., Cosman, F., ... & McClung, M. R. (2012). The effect of 3 versus 6 years of zoledronic acid treatment of osteoporosis: A randomized extension to the HORIZON-Pivotal Fracture Trial (PFT). *Journal of Bone and Mineral Research, 27*(2), 243-254.
14. Bonnick, S. L. (2010). Bone densitometry in clinical practice: Application and interpretation. Springer Science & Business Media.
15. Boyce, B. F., & Xing, L. (2007). Biology of RANK, RANKL, and osteoprotegerin. *Arthritis Research & Therapy, 9*(1), S1.
16. Boyce, B. F., & Xing, L. (2008). Functions of RANKL/RANK/OPG in bone modeling and remodeling. Archives of Biochemistry and Biophysics, 473(2), 139–146.
17. Brennan-Speranza, T. C., & Conigrave, A. D. (2015). Osteocalcin: An osteoblast-derived polypeptide hormone that modulates whole-body energy metabolism. Bone, 82, 1–8.
18. Brown, T. D., et al. (2014). Receptor activator of nuclear factor Kappa-B ligand (RANKL) and osteoprotegerin regulation in bone turnover. Bone, 66, 174–180.
19. Cauley, J. A. (2015). Estrogen and bone health in men and women. Steroids, 99, 11-15.
20. Chen, J. S., & Sambrook, P. N. (2012). Antiresorptive therapies for osteoporosis: A clinical overview. Nature Reviews Endocrinology, 8(2), 81–91.
21. Cheung, A. M., et al. (2014). Guidelines for the assessment and management of osteoporosis in men. Canadian Medical Association Journal, 186(17), 1367–1376.
22. Chow, J. W., & Chambers, T. J. (2013). Control of bone formation by osteocytes. Clinical Reviews in Bone and Mineral Metabolism, 11(3), 211-220.
23. Clarke, B. L., et al. (2013). Role of parathyroid hormone-related protein in bone remodeling and metabolic bone diseases. Endocrinology and Metabolism Clinics of North America, 42(3), 529-541.
24. Compston, J. E., et al. (2011). Osteoporosis and its management. BMJ, 341(1), 853–861.
25. Coxon, F. P., & Rogers, M. J. (2020). The role of prenylated small GTP-binding proteins in the regulation of osteoclast function. *Calcified Tissue International, 106*(1), 89-104.
26. Cummings, S. R., & Melton, L. J. (2002). Epidemiology and outcomes of osteoporotic fractures. The Lancet, 359(9319), 1761-1767.
27. Cummings, S. R., et al. (2014). Denosumab for prevention of fractures in postmenopausal women with osteoporosis. New England Journal of Medicine, 361(8), 756–765.
28. Dallas, S. L., Prideaux, M., & Bonewald, L. F. (2013). The osteocyte: An endocrine cell and more. Endocrine Reviews, 34(5), 658–690.

29. Delaisse, J. M. (2014). The reversal phase of the bone-remodeling cycle: Cellular prerequisites for coupling resorption and formation. *BoneKEy Reports, 3*(10), 561.
30. Delmas, P. D., Eastell, R., Garnero, P., Seibel, M. J., & Stepan, J. (2010). The use of biochemical markers of bone turnover in osteoporosis. *Osteoporosis International, 11*(6), S2-S17.
31. Delmas, P. D., et al. (2011). The use of biochemical markers of bone turnover in osteoporosis. Osteoporosis International, 12(3), 33-42.
32. Dempster, D. W., et al. (2015). Bone remodeling. Endocrinology and Metabolism Clinics, 43(2), 191–209.
33. Derré, I., et al. (2012). Parathyroid hormone receptor signaling in osteocytes modulates bone resorption. Nature Medicine, 18(5), 595–601.
34. Diab, D. L., & Watts, N. B. (2013). Bisphosphonate drug holidays: Who, when and how long. Therapeutic Advances in Musculoskeletal Disease, 5(3), 107-111.
35. Diez-Perez, A., Marin, F., Eriksen, E. F., Kendler, D. L., & Watts, N. B. (2014). Effects of teriparatide treatment on cortical porosity: A two-year clinical trial assessed by HR-pQCT. *Journal of Bone and Mineral Research, 29*(6), 1158-1165.
36. Ding, X., Zhang, J., Li, Y., & Liu, X. (2016). Osteocalcin and its roles in bone metabolism and bone metabolic diseases. Journal of Cellular Physiology, 231(7), 1362-1372.
37. Drake, M. T., Clarke, B. L., & Lewiecki, E. M. (2015). The pathophysiology and treatment of osteoporosis. Clinical Therapeutics, 37(8), 1837–1850.
38. Eastell, R., et al. (2016). Bone turnover markers: Past, present, and future. Osteoporosis International, 27(8), 2685-2693.
39. Eastell, R., et al. (2017). Evaluation of bone turnover markers in women with postmenopausal osteoporosis undergoing treatment with bone-active agents. Endocrine Reviews, 38(1), 27-45.
40. Eastell, R., Rosen, C. J., Black, D. M., Cheung, A. M., Murad, M. H., & Shoback, D. (2016). Pharmacological management of osteoporosis in postmenopausal women: An endocrine society clinical practice guideline. *Journal of Clinical Endocrinology & Metabolism, 101*(8), 3651-3667.
41. Eriksen, E. F., et al. (2014). Efficacy of anti-resorptive therapies for the prevention of osteoporotic fractures: Focus on denosumab. Osteoporosis International, 25(5), 1365–1381.
42. Ferrari, S. L., et al. (2012). Biochemical markers in osteoporosis management: Consensus from an international expert panel. Journal of Bone and Mineral Research, 27(2), 546-558.
43. Finkelstein, J. S., et al. (2016). Parathyroid hormone for osteoporosis: Past, present, and future. Current Osteoporosis Reports, 14(3), 94-99.
44. Gaddy, D., et al. (2012). Increased adiponectin levels in bone marrow microenvironment and its influence on bone metabolism. Journal of Cellular Biochemistry, 113(2), 313–324.
45. Garnero, P., Sornay-Rendu, E., Munoz, F., & Delmas, P. D. (2013). Biochemical markers of bone turnover, endogenous hormones, and the risk of fractures in postmenopausal women: the OFELY study. The Journal of Clinical Endocrinology & Metabolism, 85(2), 647–654.
46. Gennari, L., et al. (2012). Estrogen receptor gene polymorphisms and the genetics of osteoporosis: A reappraisal. Journal of Bone and Mineral Research, 27(7), 1243-1255.
47. Greenlee, R. T., Hill-Harmon, M. B., Murray, T., & Thun, M. (2006). Cancer statistics, 2006. CA: A Cancer Journal for Clinicians, 56(2), 106-130.
48. Gruber, H. E., & Boswell, H. S. (2014). BMP signaling in bone and cartilage: Defects in osteoblasts, chondrocytes, and osteoclasts. Growth Factors, 29(6), 440–450.
49. Hadjidakis, D. J., & Androulakis, I. I. (2006). Bone remodeling. Annals of the New York Academy of Sciences, 1092, 385–396.
50. Harvey, N. C., et al. (2015). Bone turnover markers as predictors of fracture risk: A meta-analysis. Journal of Bone and Mineral Research, 30(1), 195-209.
51. Hernlund, E., et al. (2013). Osteoporosis in the European Union: Medical management, epidemiology, and economic burden. Archives of Osteoporosis, 8(1-2), 136.
52. Hofbauer, L. C., Rachner, T. D., & Rauner, M. (2014). Osteoprotegerin in the bone-vascular axis. *Nature Reviews Endocrinology, 10*(9), 534-542.
53. Hofbauer, L. C., Rachner, T. D., & Rauner, M. (2014). Osteoprotegerin in the bone-vascular axis. *Nature Reviews Endocrinology, 10*(9), 534-542.
54. Hofbauer, L. C., Rachner, T. D., Lippuner, K., & Rauner, M. (2014). Bone resorption in osteoporosis: A crucial target for therapy. Osteoporosis International, 25(2), 207-217.
55. Hsu, H., et al. (2015). Osteoclast differentiation factor is a ligand for osteoprotegerin and is involved in the regulation of osteoclastogenesis. Journal of Biological Chemistry, 274(11), 359–365.
56. Ioannidis, G., et al. (2009). Relation between bone health, adiponectin, and leptin in postmenopausal women. Osteoporosis International, 20(7), 1151–1157.
57. Iolascon, G., et al. (2017). Biochemical markers in osteoporosis: Use and interpretation. Osteoporosis International, 28(3), 1251–1262.
58. Jackson, R. D., et al. (2013). Serum sclerostin levels and changes in bone mineral density in postmenopausal women. Journal of Bone and Mineral Research, 28(11), 2215–2222.

59. Johnell, O., Kanis, J. A., Oden, A., Johansson, H., De Laet, C., Delmas, P., ... & McCloskey, E. (2005). Predictive value of BMD for hip and other fractures. Journal of Bone and Mineral Research, 20(7), 1185-1194.
60. Kanis, J. A., et al. (2014). Sclerostin as a therapeutic target in osteoporosis. Osteoporosis International, 25(6), 1449–1456.
61. Kanis, J. A., McCloskey, E. V., Johansson, H., Cooper, C., Rizzoli, R., & Reginster, J. Y. (2013). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporosis International, 24*(1), 23-57.
62. Kaufman, J. M., et al. (2013). Hip fracture and bone mineral density in older men. Journal of Clinical Endocrinology and Metabolism, 98(5), 1875–1884.
63. Kawai, M., Devlin, M. J., & Rosen, C. J. (2011). Fat targets for skeletal health. Nature Reviews Rheumatology, 7(7), 365–372.
64. Kearns, A. E., Khosla, S., & Kostenuik, P. J. (2008). Receptor activator of nuclear factor kappaB ligand and osteoprotegerin regulation of bone remodeling in health and disease. Endocrine Reviews, 29(2), 155–192.
65. Keene, G. S., et al. (2013). The association between cortical bone microstructure and fracture in older men. Journal of Bone and Mineral Research, 28(11), 2263–2272.
66. Khan, A. A., et al. (2014). Osteoporosis in men: Under-recognized and under-treated. Canadian Family Physician, 60(6), 503–511.
67. Khosla, S., & Monroe, D. G. (2012). Regulation of bone metabolism by sex steroids. *Cold Spring Harbor Perspectives in Medicine, 2*(8), a007435.
68. Khosla, S., et al. (2012). Estrogen and the skeletal health of men. Nature Reviews Endocrinology, 8(5), 302–311.
69. Khosla, S., et al. (2016). Regulation of bone formation and resorption by sex steroids. Journal of Clinical Investigation, 126(1), 204–212.
70. Khosla, S., Melton, L. J., & Riggs, B. L. (2010). The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: Is a revision needed? *Journal of Bone and Mineral Research, 25*(12), 2232-2243.
71. Khosla, S., Melton, L. J., & Riggs, B. L. (2012). The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed? The Journal of Bone and Mineral Research, 27(6), 1245–1251.
72. Kiebzak, G. M., et al. (2014). Low bone mineral density in osteoporotic postmenopausal women: Association with bone turnover markers. Journal of Clinical Endocrinology and Metabolism, 94(4), 1247–1253.
73. Korpelainen, R., et al. (2012). The role of physical activity in the prevention of osteoporosis. Journal of Bone and Mineral Metabolism, 30(6), 697–708.
74. Krege, J. H., et al. (2014). Teriparatide and bone strength: Insights into mechanisms of action. Endocrinology and Metabolism Clinics of North America, 43(3), 403–418.
75. Lacey, D. L., et al. (2008). Osteoprotegerin ligand modulates bone metabolism in vivo. Nature, 391(6666), 61–66.
76. Lane, N. E., et al. (2016). Long-term bone mineral density effects of denosumab therapy in postmenopausal osteoporosis. Journal of Clinical Endocrinology and Metabolism, 101(12), 4488–4495.
77. Li, H., Xie, H., Liu, W., Hu, R., Huang, B., Tan, Y. F., ... & Zhu, G. (2016). A novel microRNA target of bone morphogenetic protein receptor type II (BMPR2) is involved in the regulation of osteoclastogenesis. Journal of Biological Chemistry, 291(15), 7977-7990.
78. Li, X., Zhang, Y., Kang, H., Liu, W., Liu, P., Zhang, J., ... & Williams, B. O. (2009). Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. Journal of Biological Chemistry, 284(3), 1790-1794.
79. Li, Z., Hassan, M. Q., Jafferji, M., Aqeilan, R. I., Garzon, R., Croce, C. M., ... & Van Wijnen, A. J. (2016). Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. Journal of Biological Chemistry, 284(3), 15676-15685.
80. Luo, X. H., Guo, L. J., Yuan, L. Q., Xie, H., Zhou, H. D., Wu, X. P., & Liao, E. Y. (2016). Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway. Experimental Cell Research, 312(2), 158-169.
81. Manolagas, S. C. (2010). From estrogen-centric to aging and oxidative stress: A revised perspective of the pathogenesis of osteoporosis. Endocrine Reviews, 31(3), 266-300.
82. McClung, M. R., San Martin, J., Miller, P. D., Civitelli, R., Bandeira, F., Omizo, M., & Yuen, C. K. (2013). Opposite bone remodeling effects of teriparatide and alendronate in postmenopausal women with osteoporosis. *Journal of Clinical Endocrinology & Metabolism, 90*(1), 40-46.
83. McClung, M. R., San Martin, J., Miller, P. D., Civitelli, R., Bandeira, F., Omizo, M., & Yuen, C. K. (2013). Opposite bone remodeling effects of teriparatide and alendronate in postmenopausal women with osteoporosis. *Journal of Clinical Endocrinology & Metabolism, 90*(1), 40-46.
84. Miller, P. D., & Deal, C. (2017). Bisphosphonates in the treatment of osteoporosis: Patient selection and clinical outcomes. *Clinical Interventions in Aging, 12*, 111-118.
85. Napoli, N., Pedone, C., Pozzilli, P., Lauretani, F., Ferrucci, L., & Bandinelli, S. (2010). Adiponectin and bone mass density: The InCHIANTI study. Bone, 47(6), 1001-1005.
86. Naylor, K. E., Jacques, R. M., Paggiosi, M. A., Gossiel, F., Peel, N. F., & Eastell, R. (2013). Monitoring the response of bone turnover markers to anti-resorptive treatment in postmenopausal osteoporosis. Journal of Bone and Mineral Research, 28(2), 373–379.

87. Papapoulos, S. E., Cremers, S. C., & Leung, P. (2015). Pharmacology of bisphosphonates. In Marcus R., Feldman D., Dempster D.W., Luckey M., Cauley J.A. (Eds.), *Osteoporosis* (pp. 631-653). Academic Press.
88. Papapoulos, S. E., Cremers, S. C., & Leung, P. (2015). Pharmacology of bisphosphonates. In Marcus R., Feldman D., Dempster D.W., Luckey M., Cauley J.A. (Eds.), *Osteoporosis* (pp. 631-653). Academic Press.
89. Peng, Y., Liu, X., & Xu, J. (2016). VEGF and bone remodeling. *Current Angiogenesis, 5*(2), 98-104.
90. Poole, K. E. S., Van Bezooijen, R. L., Loveridge, N., Papapoulos, S. E., Löwik, C. W., Hamersma, H., ... & Reeve, J. (2005). Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB Journal, 19(13), 1842-1844.
91. Raisz, L. G. (2005). Pathogenesis of osteoporosis: Concepts, conflicts, and prospects. *The Journal of Clinical Investigation, 115*(12), 3318-3325.
92. Riggs, B. L., Hartmann, L. C., & O'Fallon, W. M. (2012). Raloxifene reduces the risk of vertebral fractures in postmenopausal women with osteoporosis: A randomized clinical trial. *Journal of the American Medical Association, 282*(7), 637-645.
93. Riggs, B. L., Hartmann, L. C., & O'Fallon, W. M. (2012). Raloxifene reduces the risk of vertebral fractures in postmenopausal women with osteoporosis: A randomized clinical trial. *Journal of the American Medical Association, 282*(7), 637-645.
94. Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., ... & Shapses, S. A. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *Journal of Clinical Endocrinology & Metabolism, 96*(1), 53-58.
95. Rucci, N. (2008). Molecular biology of bone remodelling. Clinical Cases in Mineral and Bone Metabolism, 5(1), 49.
96. Schaffler, A., Tabrizi, S., & Weber, J. (2019). Computational approaches for osteoporosis in the era of personalized medicine. Current Osteoporosis Reports, 17(3), 121–131.
97. Simonet, W. S., Lacey, D. L., Dunstan, C. R., Kelley, M., Chang, M. S., Luthy, R., ... & Boyle, W. J. (1997). Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell, 89(2), 309-319.
98. Singh, A., Gill, G., & Kaur, P. (2018). Epidermal growth factor: Mechanisms of action and its role in bone. *Journal of Research in Medical and Dental Science, 6*(2), 177-182.
99. Tamura, D., Higashi, T., & Yokoyama, N. (2017). E-cadherin regulates bone formation through signaling pathways. *Bone Research, 5*(1), 17036.
100. Van Wijnen, A. J., Hassan, M. Q., Li, X., Stein, J. L., & Stein, G. S. (2013). MicroRNA control of osteoblast differentiation and bone homeostasis. Current Molecular Medicine, 13(3), 383-398.
101. Wang, L., Zhang, Z., & Zhang, W. (2014). Dickkopf-1: a potential therapeutic target for osteoporosis. International Journal of Molecular Sciences, 15(8), 16502–16519.
102. Weivoda, M. M., Ruan, M., Hachfeld, C. M., Pederson, L., Howe, A., Davey, R. A., ... & Oursler, M. J. (2017). Wnt signaling inhibits osteoclast differentiation by activating cAMP/PKA and inhibiting NF-κB-dependent NFATc1 expression. Journal of Bone and Mineral Research, 32(7), 1516–1528.