RESEARCH ARTICLE

Elevated Sclerostin and P1NP Levels are Associated with Osteomalacia, Particularly Among Female Patients

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Abstract

BACKGROUND: Osteomalacia is a bone metabolic disease resulting from inadequate mineralization due to vitamin D deficiency and disturbances in calcium and phosphate metabolism. To date, information on the combined assessment of traditional markers of mineral metabolism and bone turnover biomarkers in patients with osteomalacia remains scarce. Therefore, the present investigation was conducted to assess the diagnostic value of biochemical and bone turnover markers for osteomalacia and to identify the potential gender-specific differences.

METHODS: This case–control study included 100 subjects with osteomalacia and 100 healthy controls. Blood samples were collected, and serum levels of calcium, phosphate, and alkaline phosphatase (ALP) were measured using chemistry analyzer; vitamin D were and parathyroid hormone (PTH) were measured using immunofluorescence; while procollagen type 1 N-propeptide (P1NP), C-terminal telopeptide (CTX) and sclerostin level were measured using enzyme linked immunosorbent assay (ELISA). The diagnostic utility of the evaluated biomarkers was then subsequently assessed.

RESULTS: Serum calcium, phosphate, and vitamin D were significantly reduced, while ALP and PTH concentrations were increased in osteomalacia subjects compared to control (p<0.001). Sclerostin concentrations were significantly higher in osteomalacia subjects than controls (p<0.003), especially in females than in males (p=0.021). P1NP levels were significantly altered in osteomalacia subjects compared with controls (p<0.001). Biochemical profiles were comparable across genders, except for sclerostin, which was significantly higher in females (p=0.021) and lower T-scores compared with males. BMI increased significantly with age (p=0.024). ROC analysis showed strong discriminatory ability of evaluated biomarkers within the study population.

CONCLUSION: Elevated sclerostin and P1NP levels were associated with osteomalacia and may be useful biomarkers reflecting impaired bone formation, improving diagnostic accuracy when used alongside conventional markers. Sclerostin concentrations were considerably higher in female patients than in males, suggesting possible sex-related differences in bone metabolism.

KEYWORDS: sclerostin, vitamin D, osteomalacia, mineralization, bone turnover biomarkers

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Introduction

Osteomalacia is a common Oste metabolic disorder among older adults.(1) It is typically caused by a vitamin D deficiency, leading to inadequate mineralization of the osteoid matrix in both cortical and trabecular bones, resulting in osteoid buildup.(2) Globally, nutritional osteomalacia mainly results from insufficient vitamin D intake. Low vitamin D impairs the intestines' ability to absorb calcium and phosphate, which can trigger secondary hyperparathyroidism, increased bone turnover, phosphate deficiency, and a gradual accumulation of unmineralized osteoid tissue.(3) Clinically, these problems show up as

constant bone pain, muscular weakness, skeletal deformities, and insufficiency fractures, which are commonly mistaken for osteoporosis or long-term rheumatologic illnesses. Prior research has mostly focused on vitamin D insufficiency and traditional biochemical measures; however, there is little, and often conflicting, information about how these indicators behave in osteomalacia. Specifically, it has been difficult to determine how sex affects how they manifest. To better understand their diagnostic and pathophysiological implications in osteomalacia, more research is therefore required.(4)

In biochemistry, osteomalacia is typically associated with low blood levels of 25-hydroxyvitamin D, elevated alkaline phosphatase, altered calcium and phosphate levels, and high parathyroid hormone levels.(5) Because comparable changes may also occur in other metabolic bone disorders, such as osteoporosis, familial hypophosphatemic syndromes, and chronic kidney disease–mineral and bone disorder (CKD-MBD), thus determining the specific diagnostic value of these biomarkers remains challenging. (6) Osteomalacia can also happen when the kidneys are ill or stop working. Getting rid of phosphorous from the blood is a highly vital job for the kidneys. Phosphorus can build up if the kidneys don't work appropriately. The kidneys also convert vitamin D into calcitriol, the form that is active. Calcitriol helps maintain calcium and phosphorus levels in the blood. When the kidneys cease functioning, phosphorus levels rise and active vitamin D levels fall. This causes the body to produce more parathyroid hormone, leading to hyperparathyroidism. High levels of parathyroid hormone help keep blood phosphorus levels in balance by promoting calcium release from bones. Bone calcium might be lost, making them weak and brittle.(7)

The liver plays a role in vitamin D metabolism, and its malfunction can lead to osteomalacia due to abnormal calcium and phosphate balance and impaired bone mineralisation.(8–10) Bone remodelling is a process in which bone growth and resorption are balanced. Some bone turnover markers are procollagen type 1 N-propeptide (P1NP), which is a marker of osteoblastic bone production, and sclerostin, which can inhibits the Wnt/β-catenin pathway and bone formation. Interest in metabolic bone disorders has been increasing, but the diagnostic value of these bone turnover markers for osteomalacia remains poorly understood. Most investigations have focused on vitamin D deficiency and traditional biochemical markers, but little is known about the impact of P1NP and sclerostin. Further studies are therefore needed to elucidate their clinical value in diagnosing and assessing osteomalacia.(11,12)

Despite the growing interest in metabolic bone disorders, information on the combined assessment of traditional markers of mineral metabolism and bone turnover biomarkers in patients with osteomalacia is scarce. Moreover, the correlations between these biomarkers and demographic parameters such as age and sex are yet to be well characterized. Therefore, the purpose of this study was to evaluate the biochemical status of osteomalacia patients by determining the serum concentrations of vitamin D, calcium, phosphorus, parathyroid hormone (PTH), alkaline phosphatase (ALP) and bone turnover markers, including P1NP, C-terminal telopeptide (CTX) and sclerostin. Furthermore, the study studied the impact of demographic factors on these biomarkers and their potential usefulness in indicating disease severity and bone remodelling status.

Methods

Subject Recruitment

The present investigation was a case-control observational study conducted in the Clinical Biochemistry Laboratories of Al-Fayhaa Teaching Hospital, Basra Teaching Hospital and Ports General Hospital in Basra City, Iraq in collaboration with the Rheumatology and Endocrinology Clinics. The study took place between August 2025 and February 2026. All patients were examined and diagnosed by specialized physicians according to clinical symptoms, biochemical abnormalities and radiological findings when available. The clinical examination was based on symptoms suggesting osteomalacia (bone pain and muscular weakness) and biochemical criteria included abnormalities in vitamin D, calcium, phosphate, ALP, and PTH levels. Radiological examination was performed on participants with clinical symptoms suggestive of osteomalacia, which included chronic bone pain, muscle weakness, skeletal discomfort, trouble walking or a history of fragility fractures. Standard radiographic imaging was ordered by the treating physician as part of the standard clinical evaluation to uncover distinctive skeletal abnormalities associated with osteomalacia. Radiological results were evaluated and recorded for diagnostic confirmation and for association with biochemical indicators.

The inclusion criteria for participants were as follows: The osteomalacia group included individuals who had a diagnosis of osteomalacia based on the accepted clinical and biochemical criteria, with the control group being healthy volunteers who were matched based on their age and sex and were not affected by metabolic bone disorders. Subjects

were excluded if they have chronic liver disease (elevated liver function tests above the upper normal reference range), chronic kidney disease (estimated glomerular filtration rate <60 mL/min/1.73 m^2), malignancy, chronic inflammatory disorders, pregnancy or lactation, recent major trauma, surgery or fracture and current use of medications known to influence bone metabolism (*e.g.*, glucocorticoids, anticonvulsants or anti-osteoporotic drugs). The existence of these disorders was confirmed by medical history, clinical examination and, when appropriate, relevant laboratory testing. Healthy controls were recruited from hospital staff, patient attendants, and community volunteers, matched to the patient group by age and sex to the greatest extent possible. Total 200 individuals (100 osteomalacia subjects and 100 healthy controls) aged 14 to 70 were finally recruited in this study, with both groups included 50 females and 50 males.

Blood Samples Collection

From each subject, 5 mL of venous blood was collected from the antecubital vein using disposable sterile syringes. The blood was drawn into yellow gel separator tubes containing a clot activator and then left at room temperature until clot formation was complete.

Data Collection

Data were collected through a closed questionnaire that included 15 questions about concepts such as name, age, symptoms of bone turnover disease (*e.g.*, difficulty walking, numbness), other chronic diseases, thyroid disease, any bone scans or bone biopsies, medication use, place of residence, digestive disorders, height, weight, and smoking. Body mass index (BMI) was calculated by dividing weight (kg) by height (m^2).(13)

P1NP, CTX and Sclerostin Measurement Using Enzyme Linked Immunosorbent Assay (ELISA)

Serum levels of P1NP, CTX and sclerostin were determined using commercially available enzyme-linked immunosorbent assay kits, as follow: Human Procollagen Type 1 N-Terminal Propeptide ELISA Kit (Cat. No. E1350Hu; BT LAB, Shanghai, China) with range of 2.5–160 ng/mL and sensitivity of 1.15 ng/mL; Human Cross-linked C-telopeptide of Type 1 Collagen ELISA Kit, (Cat. No. E1349Hu; BT LAB) with range of 7–1500 ng/mL and sensitivity of 4.21 ng/mL; Human Sclerostin ELISA Kit (Cat. No. E3068Hu, BT LAB) range: 0.5–200ng/mL and sensitivity: 0.26ng/mL. The measurement was performed as per the manufacturer's instructions. Briefly, the assays were based on antigen–antibody binding, in which target analytes are captured by specific antibodies coated on microplates and detected with enzyme-conjugated secondary antibodies and a colorimetric substrate. The intensity of the colour created was proportional to the analyte concentration and is quantified spectrophotometrically.

Calcium, Phosphate, ALP, Vitamin D, and PTH Measurement

The clotted blood samples in yellow tubes were centrifuged at 3000 rpm for 10 minutes to obtain clear serum. The separated serum was carefully aspirated into labeled Eppendorf tubes, aliquoted for hormonal and biochemical assays, and stored at −80°C until analysis, as recommended in the study proposal, to avoid repeated freeze–thaw cycles. Biochemical parameters including calcium, phosphate, ALP were measured by using manual chemistry analyzer. Meanwhile 25 hydroxyvitamin D and PTH level were measured by using immunofluorescence analyzer (Microprofit Biotech, Shenzhen, China).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 27.0 (IBM Corporation, Armonk, NY, USA), was used to perform statistical analyses. Categorical variables were displayed as frequencies and percentages, whereas continuous variables were represented as mean±standard deviation (SD). Comparisons of continuous variables across the three study groups were performed using One-Way Analysis of Variance (ANOVA), and independent-samples t-tests were used to compare two independent groups. The Chi-square test was used to analyze categorical data. Relationships among biochemical markers were evaluated using the Pearson correlation coefficient. Additionally, Receiver Operating Characteristic (ROC) curve analysis was performed to assess the diagnostic accuracy of the parameters. A p-value≤0.05 was considered statistically significant.

Results

Biochemical Variables Estimation

The demographic characteristics of all subjects (100 patients and 100 healthy controls) were shown in Table 1. The present investigation showed that osteomalacia patients had significantly lower serum calcium, phosphate and vitamin D levels with significantly higher ALP, P1NP, CTX, sclerostin and PTH levels as compared to healthy controls.

Table 1. Demographic and biochemical characteristics of osteomalacia and control subjects.

Variables	Osteomalacia (n=100)	Controls (n=100)	*p*-value
BMI (kg/m²)	24.79±2.58	24.72±2.15	0.862
Ages (years)	45.51±14.71	45.73±10.92	0.915
Gender %, n (%)			
Male	36 (48.0%)	35 (46.7%)	
Female	39 (52.0%)	40 (53.3%)	
T-score	-1.82±0.46	-0.02±0.33	<0.001*
Calcium (mg/dL)	7.86±0.39	9.62±0.31	<0.001*
Phosphate (mg/dL)	1.94±0.30	3.93±0.25	<0.001*
ALP (U/L)	182.61±24.45	63.37±7.22	<0.001*
Vitamin D (ng/mL)	8.77±1.31	38.83±4.32	<0.001*
PTH (pg/mL)	160.97±30.08	35.56±6.55	<0.001*
P1NP (ng/mL)	178.61±46.29	43.37±13.73	0.008*
CTX (ng/mL)	1.73±0.20	0.31±0.12	0.021*
Sclerostin (ng/mL)	1.52±0.17	0.37±0.12	<0.005*

Values are expressed as mean±SD. Continuous variables were compared between patients and controls with an independent samples t-test and gender distribution was compared using a Chi-square test. Statistical significance was set at $p<0.05$.

Gender-Specific Differences in T-score, P1NP, CTX, Sclerostin and Vitamin D Levels

There was a modest sex-related variations among individuals with osteomalacia for most biochemical markers (Table 2). Significant differences were identified in T-score, P1NP, CTX, sclerostin and vitamin D between males and females ($p>0.05$). This finding suggested that the metabolic abnormalities associated with osteomalacia were mainly related to disease status rather than sex differences. Meanwhile, in the control group, there were no statistically significant differences between the sexes across all assessed parameters ($p>0.05$). These data demonstrate similar demographic and biochemical characteristics of male and female controls. This supports the view that the control group was homogeneous and can be compared with osteomalacia cases.

Age-Specific Differences in P1NP and Vitamin D Levels

There was a significant increase in the BMI of osteomalacia patients with advancing age (Table 3). The highest values were observed in people aged 50 and older (25.50±1.90 kg/m²), whereas younger groups had lower values ($p=0.024$). This finding could be because older people are less active, their metabolisms slow with age, and they have more fat mass, since all of these parameters were known to impair vitamin D bioavailability and bone health. P1NP and vitamin D showed a significant difference between the ages; on the other hand, serum calcium, phosphorus, ALP, vitamin D, PTH, sclerostin, and CTX did not shown to be statistically significant differences across age groups ($p>0.05$). For all indicators in the controls, there was no statistically significant difference in age ($p>0.05$), which means that people in the controls were about the same age.

Table 2. Comparison of mean values of the studied biomarkers by sex in the osteomalacia patient group.

Variables	Osteomalacia			Controls		
	Male (n=50)	Female (n=50)	*p*-value	Male (n=50)	Female (n=50)	*p*-value
BMI (kg/m²)	25.20±2.57	24.41±2.57	0.192	25.46±1.90	24.1±2.16	0.834
Ages (years)	42.78±15.29	48.03±13.87	0.125	47.52±10.68	44.24±11.02	0.299
T-score	-1.38±0.18	-2.23±0.16	<0.001*	-0.029±0.324	-0.012±0.346	0.820
Calcium (mg/dL)	7.76±0.42	7.96±0.34	0.023*	9.614±0.297	9.626±0.317	0.536
Phosphate (mg/dL)	1.93±0.29	1.95±0.30	0.757	3.964±0.214	3.907±0.278	0.841
ALP (U/L)	185.69±24.31	179.77±24.54	0.297	63.5±6.94	63.26±7.51	0.072
Vitamin D (ng/mL)	8.58±1.24	8.01±1.37	0.008*	38.61±3.68	39.01±4.82	0.147
PTH (pg/mL)	155.92±29.21	166.44±30.45	0.132	36.41±5.93	34.85±7.00	0.207
P1NP (ng/mL)	145.21±32.55	209.45±34.14	0.010*	43.47±14.05	43.28±13.62	0.570
CTX (ng/mL)	1.55±0.04	1.91±0.12	0.032*	0.313±0.119	0.301±0.115	0.740
Sclerostin (ng/mL)	1.69±0.04	1.36±0.03	0.021*	0.366±0.125	0.370±0.121	0.361

Values are expressed as mean±SD. Continuous variables were compared between patients and controls with an independent samples t-test and gender distribution was compared using a Chi-square test. Statistical significance was set at $p<0.05$.

Table 3. Analysis of all biomarkers in patients according to the age of osteomalacia patients.

Variables	Osteomalacia				Controls			
	<25 Year (n=20)	25-50 Year (n=40)	>50 Year (n=40)	*p*-value	<25 Year (n=20)	25-50 Year (n=40)	>50 Year (n=40)	*p*-value
BMI (kg/m²)	22.26±2.09	24.56±2.78	25.78±1.84	0.002	21.93±0.37	24.69±2.26	25.11±1.78	0.050
T-score	-1.66±0.46	-1.82±0.44	-1.87±0.49	0.537	-0.333±0.37	-0.006±0.34	-0.008±0.30	0.256
Calcium (mg/dL)	7.92±0.32	7.87±0.45	7.83±0.34	0.814	9.73±0.35	9.64±0.29	9.55±0.32	0.388
Phosphate (mg/dL)	1.95±0.15	1.89±0.37	2.00±0.20	0.310	3.76±0.37	3.92±0.24	3.96±0.24	0.419
ALP (U/L)	183.50±25.51	179.37±24.66	186.62±24.13	0.488	65.33±6.02	62.89±7.47	64.08±6.98	0.722
Vitamin D (ng/mL)	9.07±1.37	8.74±1.26	7.85±0.98	0.063	40.03±2.68	39.2±4.47	37.95±4.15	0.459
PTH (pg/mL)	156.76±32.64	161.95±28.63	171.62±27.56	0.452	31.66±0.57	35.34±6.54	36.22±6.81	0.495
P1NP (ng/mL)	188.86±31.56	180.65±43.06	131.85±76.95	0.007	33.56±11.62	43.19±14.02	44.55±13.51	0.418
CTX (ng/mL)	1.61±0.13	1.74±0.21	1.76±0.19	0.162	0.360±0.12	0.299±0.11	0.312±0.11	0.644
Sclerostin (ng/mL)	1.50±0.17	1.51±0.17	1.58±0.16	0.564	0.382±0.14	0.370±0.11	0.365±0.13	0.963

Values are expressed as mean±SD. Continuous variables were compared between patients and controls with an independent samples t-test and gender distribution was compared using a Chi-square test. Statistical significance was set at $p<0.05$.

High Diagnostic Accuracy of Sclerostin and P1NP

ROC analysis was used to determine the optimal specificity and sensitivity for a diagnostic test. This was achieved by using a figure that demonstrates the correlation between specificity and sensitivity. The ROC curve research had revealed the precise cut-off values for the blood biomarkers that effectively differentiate between osteomalacia and healthy conditions. Figure 1 displayed the curve and area under the curve (AUC) analysis for the serum biomarker values used to diagnose osteomalacia.

Logistic Regression Analysis for Osteomalacia Subjects

Logistic regression analysis was used to evaluate whether the studied biomarkers (*e.g.*, vitamin D, PTH, calcium) were independent predictors of osteomalacia. It assesses the effect of all variables simultaneously to determine which factors significantly influence disease risk. In this study, Logistic regression analysis showed that lower calcium, phosphorus, ALP, and vitamin D levels were significantly associated with osteomalacia, whereas higher PTH, P1NP, CTX, and sclerostin levels were associated with increased disease risk (Table 4, Figure 2).

Correlation Study between Biochemical Variables

Pearson correlation analysis showed multiple biologically relevant connections between mineral metabolism and bone turnover indicators in patients with osteomalacia. Vitamin D was strongly positively correlated with serum calcium (r=0.79) and phosphate (r=0.63) and negatively correlated with PTH (r=-0.85) and ALP (r=-0.77). Moreover, PTH was substantially and positively associated with ALP (r=0.90) and negatively associated with calcium (r=-0.86). These findings were consistent with the abnormal mineral homeostasis and compensatory secondary hyperparathyroidism typical of

Table 4. Identification of risk of incident osteomalacia by multivariable logistic regression.

Variables	Regression Coef.	Std. Error	Wald	*p*-value	Odds Ratio	95% CI Lower	95% CI Upper
Calcium (mg/dL)	-2.090	0.650	10.34	0.05	0.238	0.034	0.442
Phosphate (mg/dL)	-2.352	0.552	18.155	0.041	0.156	0.032	0.280
ALP (U/L)	-0.046	0.011	17.489	0.05	0.955	0.936	0.973
Vitamin D (ng/mL)	-0.166	0.036	21.262	0.011	0.849	0.788	0.908
PTH (pg/mL)	0.328	0.105	9.758	0.02	1.415	1.141	1.688
P1NP (ng/mL)	1.008	0.402	6.287	0.006	3.626	1.251	6.001
CTX (ng/mL)	1.009	0.350	8.310	0.005	3.417	1.382	5.451
Sclerostin (ng/mL)	0.651	0.213	9.341	0.03	1.902	1.263	2.652

Statistical analysis was performed using binary logistic regression. Wald χ^2 test was used to assess the significance of regression coefficients. Odds ratios (ORs) are presented with 95% confidence intervals (95% CI).

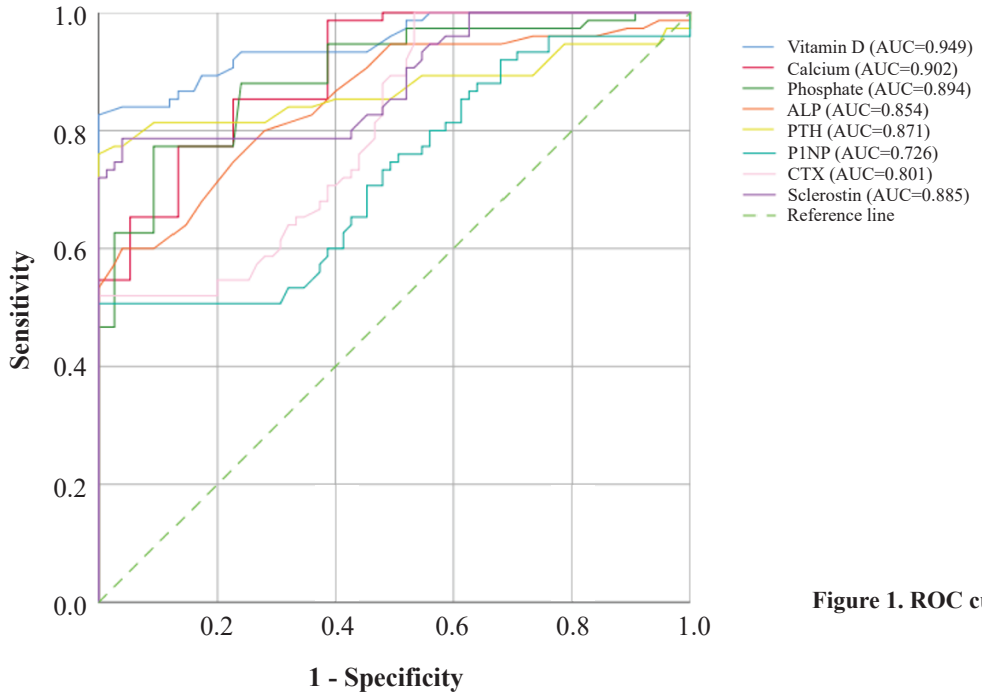

Figure 1. ROC curves for osteomalacia diagnosis.

osteomalacia. Figure 3 displayed the correlation heatmap of the osteomalacia subjects to see the most biologically feasible and therapeutically meaningful correlations.

Discussion

The results of current study are consistent with the typical biochemical profile of osteomalacia and suggest alterations in mineral metabolism and bone remodeling, including impaired bone mineralization.(14) Elevated ALP and PTH values may indicate increased osteoblastic activity and secondary hyperparathyroidism due to vitamin D insufficiency. Comparable biochemical changes have been previously described in nutritional osteomalacia, with decreased levels of vitamin D, calcium, and phosphate, and increased levels of ALP and PTH.(15) One of the remarkable findings of the present investigation was the significantly higher serum sclerostin levels in osteomalacia patients in comparison with controls. Sclerostin is a protein produced by osteocytes and is involved in regulating bone growth and bone metabolism. Previous studies have shown that altered sclerostin levels are associated with poor bone metabolism and reduced mineralization capacity.(16) Previous studies have also reported associations between vitamin D deficiency and elevated sclerostin levels, supporting the present study's findings.(17) In addition, the levels of P1NP and CTX were considerably higher (p=0.008 and p=0.021, respectively), which indicates that osteomalacia patients have more bone turnover activity. High levels of these bone turnover indicators may indicate alterations in the processes of bone formation and resorption and are associated with poor mineralization.(18)

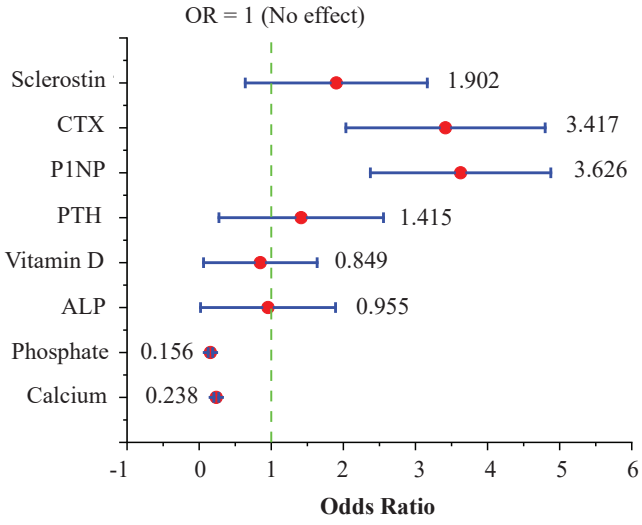

Figure 2. Logistic regression odds ratios (95% CI) for osteomalacia.

The concomitant increase of both P1NP as bone formation marker and CTX as bone resorption marker in this study is indicative of high bone turnover. This is often driven by secondary hyperparathyroidism in the setting of disturbed mineral metabolism, such as vitamin D deficiency

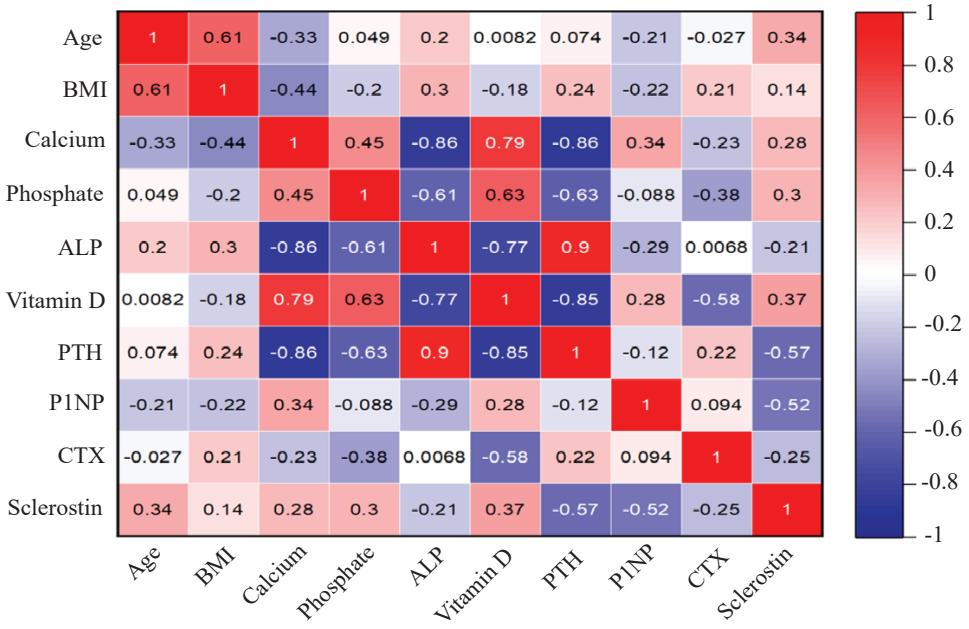

Figure 3. Correlation heatmap for osteomalacia subjects.

or osteomalacia. Increased PTH induces osteoblast-mediated production of Receptor activator of nuclear factor kappa-B ligand (RANKL) and inhibits osteoprotegerin (OPG), leading to increased osteoclast differentiation and bone resorption, indicated by increased CTX levels. Of note, bone remodeling is a linked physiological process, such that increased resorption is accompanied by compensatory activation of osteoblasts, which is reflected in enhanced bone formation markers such as P1NP.(19)

In the sex effect, female patients had considerably lower T-scores, calcium and vitamin D levels than male patients, which indicates a higher impairment in bone mineralization. Serum level of sclerostin was substantially greater in females than in males (31.48±8.94 *vs.* 25.76±9.77 pg/mL, p=0.021). This finding may reveal differences in bone metabolic control between males and females and may reflect variations in osteocyte-related activity associated with osteomalacia.(20) Significant difference of P1NP and CTX was observed between the two groups, indicating approximately equal bone turnover activity of male and female patients. Additionally, BMI, phosphorus, ALP, and PTH showed no significant differences by sex, suggesting similar alterations in mineral metabolism in both groups. This probably because the metabolic abnormalities associated with osteomalacia were mainly related to disease status rather than sex differences.(21,22)

In the present investigation, serum sclerostin levels were substantially higher in female patients than in male patients. This observation may be related to the age-associated hormonal changes and this is especially so because the mean age of female patients was in the perimenopausal phase. Previous investigations have shown that estrogen may regulate sclerostin expression and osteocyte function and then influence bone formation and mineralization process.(23) Increased sclerostin levels have been associated with reduced osteoblastic activity by regulating the Wnt/β-catenin signaling pathway, which may lead to reduced bone production. In osteomalacia, where faulty mineralization is already present, increased sclerostin levels may further suggest abnormalities in the regulation of bone metabolism. Recently, it has also been reported that hormonal changes occurring in menopause can affect osteokine production and bone quality in women.(24) Thus, the elevated sclerostin levels in female osteomalacia patients may be due to the combined impact of hormonal influences and metabolic bone abnormalities on bone remodeling activities. These findings may help to understand the more severe skeletal damage in female patients than in males.(25) In conclusion, these results demonstrate that osteomalacia has a similar effect on both sexes in most biochemical parameters, but female patients with osteomalacia may have a worse impairment of bone mineralization and skeletal status than males.

The variation in age-related biomarkers indicated that the metabolic characteristics of osteomalacia are highly consistent once the disease develops. Overall, the primary metabolic disorder of defective osteoid mineralization caused by vitamin D deficiency seems to be the same across age groups.(26) Sclerostin levels were higher in older than in younger patients. This observation can also be

attributed to age-related changes in bone metabolism and osteocyte activity, as well as the metabolic consequences of osteomalacia. Other studies also showed that sclerostin levels may increase with age and be associated with decreased bone formation and altered bone turnover.(27) In this study, the BMI is higher in older people. This could be because older people are less active, their metabolisms slow with age, and they have more fat mass. And this metabolic parameters are also known to impair vitamin D bioavailability and bone health.(28)

The ROC curve study showed that most serum biomarkers had good to outstanding diagnostic performance for osteomalacia (Figure 1). Vitamin D demonstrated the best diagnostic performance (AUC = 0.949, sensitivity = 82.7%, specificity = 100%, efficiency = 91.3%), indicating strong discriminative ability between ill patients and healthy controls. The findings imply a major role for vitamin D insufficiency in metabolic bone diseases and in poor bone mineralization. Similar findings were published previously revealed that changes in vitamin D metabolism are closely correlated with skeletal abnormalities and increased bone fragility.(29)

Calcium also showed high diagnostic accuracy (AUC = 0.934, sensitivity = 90.7%, specificity = 89.3%, efficiency = 90.0%). Disruption of calcium homeostasis contributes to poor skeletal integrity and has direct effects on bone mineralisation. Calcium imbalance is one of the key biochemical changes related to osteomalacia and osteoporosis, as verified by previous investigations.(30)

Phosphate also showed good diagnostic value (AUC = 0.949, sensitivity = 82.7%, specificity = 100%, efficiency = 91.3%). Hypophosphatemia is thought to be a primary cause of poor mineralisation, because phosphate is important for hydroxyapatite production and bone matrix integrity. Phosphate insufficiency has been linked to impaired normal bone metabolism and a dramatically increased risk for fracture.(31)

PTH showed excellent diagnostic performance (AUC = 0.861, sensitivity = 80.0%, specificity = 82.7%, efficiency = 81.3%). If PTH is high, it may indicate secondary hyperparathyroidism due to vitamin D deficiency and calcium imbalance. Increased PTH induces bone resorption and increases skeletal turnover, contributing to disease progression.(32)

ALP also had a good diagnostic accuracy (AUC = 0.836, sensitivity = 74.7%, specificity = 85.3%, efficiency = 80.0%). Higher ALP activity indicates higher osteoblastic activity and less mineralization. ALP has been proposed as a sensitive biochemical diagnostic in metabolic bone disorders such as osteomalacia.(33) The bone turnover markers CTX (AUC = 0.801, sensitivity = 76.0%, specificity = 74.7%, and efficiency = 75.3%) and P1NP (AUC = 0.814, sensitivity = 77.3%, specificity = 76.0%, and efficiency = 76.7%) showed intermediate diagnostic performance. These biomarkers reflect bone resorption and bone formation, but their levels are influenced by physiological parameters such as age, gender, renal function, and circadian variation, which can limit their discriminative accuracy when used individually. (34) The sclerostin has a very strong diagnostic ability (AUC = 0.889, sensitivity = 78.7%, specificity = 90.7%, efficiency = 84.7%). Sclerostin is an essential regulator of bone remodeling, acting to limit osteoblastic activity via the Wnt signaling pathway. Increased levels of sclerostin have been related with poor bone formation and decreased bone mass in metabolic bone disorders.(35) In conclusion, these findings show that the combination of markers of mineral metabolism with markers of bone turnover may improve the diagnostic discrimination of osteomalacia.

We noted in the logistic regression analysis that calcium and phosphorus had strong negative associations with osteomalacia, indicating poor mineralization. Increased PTH levels may reflect compensatory secondary hyperparathyroidism due to vitamin D insufficiency and altered calcium metabolism. The increase of P1NP and CTX levels in osteomalacia patients suggests the alteration of bone turnover activity and augmentation of skeletal remodeling. Higher sclerostin concentrations may be associated with altered osteocyte activity and reduced bone formation by inhibiting the Wnt/β-catenin signaling pathway. In conclusion, the present data demonstrate that changes in mineral metabolism and bone remodeling indicators are related with the pathogenesis of osteomalacia. The forest plot of logistic regression analysis showed that the relationships between the examined biomarkers and osteomalacia were inconsistent. P1NP and CTX had significantly larger positive ORs than the other biomarkers, indicating a relatively stronger statistical association with illness occurrence in the regression model. Sclerostin and PTH were also positively associated, whereas calcium, phosphorus, ALP, and vitamin D were associated with odds ratios <1, indicating inverse correlations with the prevalence of osteomalacia.

These data are generally consistent with the ROC analysis, which revealed good diagnostic performance for indicators of mineral metabolism, particularly vitamin D, calcium, and phosphorus. The differences observed between ROC and logistic regression analyses may be related to the distinct statistical purposes of each method: ROC analysis

evaluates discriminative ability, whereas logistic regression assesses the independent contribution of each biomarker after adjustment for other variables. Taken together, the results indicate that anomalies of mineral metabolism and altered bone turnover indicators are related to osteomalacia.(36)

Correlation analysis was further conducted as it has been deemed a useful statistical tool for evaluating interactions among vitamin D, PTH, calcium, phosphate, and bone remodelling indicators in metabolic bone diseases.(37) In the correlation between the biomarkers, a substantial negative association was found between vitamin D and PTH, indicating an adverse link between vitamin D status and parathyroid activity. Negative associations between calcium, phosphate, and PTH also suggested abnormalities in mineral homeostasis associated with osteomalacia. These findings are consistent with earlier research reporting that vitamin D deficiency influences calcium homeostasis and that vitamin D insufficiency is associated with increased PTH production to maintain serum calcium levels.(38,39)

The P1NP metric was positively correlated with parameters associated with bone production, indicating elevated bone turnover activity in patients with osteomalacia. On the other hand, CTX correlated less strongly with the biomarkers studied. This could indicate some diversity in bone resorption activity among patients. Sclerostin showed variable correlations with many biochemical indicators, supporting the hypothesis that sclerostin may be involved in changes in bone metabolism. Sclerostin is an osteocyte-derived protein which has been shown to regulate the Wnt/β-catenin signalling pathway and may influence bone formation.(40)

Overall, the results of the heatmap indicate that hypovitaminosis D, increased PTH, a disturbed calcium/phosphate balance and osteocyte signaling pathways are tightly linked in the etiology of osteomalacia and osteoporosis. The observed associations corroborate current hypotheses that characterize sclerostin and Wnt signaling as essential regulators of skeletal remodeling and potential indicators of metabolic bone disease progression.

The study has some limitations, including the cross-sectional design, relatively small sample size, and enrollment from a single site, which could limit the generalizability of the findings. Also, external validation of the diagnostic models was not undertaken. Larger multicenter cohorts and longitudinal studies should be conducted in the future to validate these findings and further evaluate the clinical value of the examined biomarkers in osteomalacia. Moreover, although numerous biomarkers demonstrated good diagnostic performance, the possibility of overestimation cannot be ruled out given sample characteristics and the lack of external validation. Thus, these results need to be validated in larger independent cohorts before clinical adoption.

Conclusion

The present investigation showed remarkable changes in mineral metabolism and bone turnover indicators in patients with osteomalacia. Mineral homeostasis was disturbed, as evidenced by decreased vitamin D, calcium, and phosphate levels and increased PTH and ALP concentrations. Impaired bone mineralization was seen. Furthermore, osteomalacia patients had increased levels of P1NP, CTX, and sclerostin, suggesting alterations in bone turnover activity compared to controls. Our results show that sclerostin and P1NP might be viable biomarkers for assessing osteomalacia, but prospective multicentric studies with larger sample sizes are required to confirm their clinical value before they can be used in routine clinical practice.

Authors Contribution

EQJ was involved in concepting and planning the research, RGM performed the data acquisition/collection and data analysis. EQJ interpreted the study result, prepared the manuscript draft, and designed figure and table. RGM and EQJ took parts in giving critical revision of the manuscript.

Ethical Statement

Ethical approval was obtained from the Training and Human Development Center at the Al-Basrah Health Department (No. 592/2025, dated 18/9/2025). Additionally, the Scientific Committee of the University of Basra's Pathological Analyses Department approved the study, in accordance with international ethical guidelines for research involving human health, in collaboration with WHO and CIOMS. All participants, both patients and healthy controls, gave written informed consent. Also, all human-contact procedures discussed in this study received approval from the University of Basrah's Human and Animal Ethics Committee in Iraq (No. 2025/201).

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Młynarska E, Lisińska W, Hossa K, Krupińska N, Jakubowska P, Rysz J, *et al.* Vitamin D and chronic disorders: A review of metabolic and cardiovascular diseases. Pharmaceuticals. 2025; 18(10): 1467. doi: 10.3390/ph18101467.
2. Azzahra BF, Yueniwati Y, Permatasari HK, Rahayu RF, Tifani AS, Fauzi A. Combined vitamin D and magnesium supplementation improves insulin, HOMA indices, blood glucose, and oxidative stress markers in diabetic rats. Indones Biomed J. 2026; 18(1): 20–7.
3. Emini-Sadiku M, Morina-Kuqi N. Concealing clothing leading to severe vitamin D deficiency, osteomalacia and muscle weakness. Open Access Maced J Med Sci. 2019; 7(13): 2146–9.
4. Cianferotti L. Osteomalacia is not a single disease. Int J Mol Sci. 2022; 23(23): 14896. doi: 10.3390/ijms232314896.
5. Hendrianingtyas M, Rachmawati B, Riwanto I, Mulyono B, Suhartono S, Winarni TI, *et al.* Parathyroid hormone-25 (OH) D and calcium-phosphorus ratio as osteopenia risk factors in women with central obesity. Indones Biomed J. 2022; 14(2): 148–55.
6. Bikle DD. Vitamin D: Newer concepts of its metabolism and function at the basic and clinical level. J Endocr Soc. 2020; 4(2): bvz038. doi: 10.1210/jendso/bvz038.
7. Khan MA, Dar HA, Baba MA, Shah AH, Singh B, Shiekh NA. Impact of vitamin D status in chronic liver disease. J Clin Exp Hepatol. 2019; 9(5): 574–80.
8. Minisola S, Fukumoto S, Xia W, Corsi A, Colangelo L, Scillitani A, *et al.* Tumor-induced osteomalacia: A comprehensive review. Endocr Rev. 2022; 44(2): 323–53.
9. Gou M, Ma Z. Osteomalacia, renal Fanconi syndrome, and bone tumor. J Int Med Res. 2018; 46(8): 3487–90.
10. Al-Daghri NM, Yakout S, Sabico S, Wani K, Hussain SD, Aljohani N, *et al.* Establishing the prevalence of osteomalacia in Arab adolescents using biochemical markers of bone health. Nutrients. 2022; 14(24): 5354. doi: 10.3390/nu14245354.
11. Fukumoto S, Yamashita T. Fibroblast growth factor (FGF)-23 and hypophosphatemic Rickets/Osteomalacia. Endocr J. 2001; 48(6): 603–10.
12. Ito N, Hidaka N, Kato H. Acquired forms of fibroblast growth factor 23-Related hypophosphatemic osteomalacia. Endocrinol Metab. 2024; 39(2): 255–61.
13. Mutaeb ZA, Ramadhan UH, Jasim EQ. Apelin, C-reactive protein, and serum protein correlation with blood pressure – A biomarker analysis. Eur J Clin Exp Med. 2025; 23(4): 947–54.
14. Meehan M, Penckofer S. The role of vitamin D in the aging adult. J Aging Gerontol. 2014; 2(2): 60–71.
15. Atteritano M, Di Mauro E, Canale V, Bruzzese AM, Ricciardi CA, Cernaro V, *et al.* Higher serum sclerostin levels and insufficiency of vitamin D are strongly associated with vertebral fractures in hemodialysis patients: a case control study. Osteoporos Int. 2016;28(2): 577–84.
16. Poole KES, Van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Löwik CW, *et al.* Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J. 2005; 19(13): 1842–4.
17. Acıbucu F, Dokmetas H, Acıbucu D, Kılıclı F, Aydemir M, Cakmak E. Effect of vitamin D treatment on serum sclerostin level. Exp Clin Endocrinol Diabetes. 2016; 125(09): 634–7.
18. Cain CJ, Rueda R, McLelland B, Collette NM, Loots GG, Manilay JO. Absence of sclerostin adversely affects B-cell survival. J Bone Miner Res. 2012; 27(7): 1451–61.
19. Eastell R, Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. Lancet Diabetes Endocrinol. 2017; 5(11): 908–23.
20. Kusuma BW, Dwiningsih SR, As'adi A, Wahyuningsih SP. Liposome-based nanoparticles encapsulating vitamin D3 attenuate IL-6 and TNF-α in a menopausal mouse model. Indones Biomed J. 2025; 17(5): 484–92.
21. Jasim EQ, Abdulqader MJ. Evaluation of serum ferritin and vitamin D and their correlation with TSH in hypothyroid patients in Basrah City. Front Health Inform. 2024; 13(3): 3239–48.
22. Minisola S, Colangelo L, Pepe J, Diacinti D, Cipriani C, Rao SD. Osteomalacia and vitamin D status: A clinical update 2020. JBMR Plus. 2020; 5(1): e10447. doi: 10.1002/jbm4.10447.
23. Roforth MM, Fujita K, McGregor UI, Kirmani S, McCready LK, Peterson JM, *et al.* Effects of age on bone mRNA levels of sclerostin and other genes relevant to bone metabolism in humans. Bone. 2013; 59: 1–6. doi: 10.1016/j.bone.2013.10.019.
24. Zárate S, Stevnsner T, Gredilla R. Role of estrogen and other sex hormones in brain aging: Neuroprotection and DNA repair. Front Aging Neurosci. 2017; 9: 430. doi: 10.3389/fnagi.2017.00430.
25. Mills EG, Yang L, Nielsen MF, Kassem M, Dhillo WS, Comninos AN. The relationship between bone and reproductive hormones beyond estrogens and androgens. Endocr Rev. 2021; 42(6): 691–719.
26. Laird E, Ward M, McSorley E, Strain JJ, Wallace J. Vitamin D and bone health; Potential mechanisms. Nutrients. 2010; 2(7): 693–724.
27. Hay JL, Zabor EC, Kumar J, Brennessel D, Kemeny MM, Lubetkin EI. Cancer beliefs and patient activation in a diverse, multilingual primary care sample. Psycho-Oncol. 2016; 25(9): 1071–8.
28. Arboleya L, Braña I, Pardo E, Loredo M, Queiro R. Osteomalacia in adults: A practical insight for clinicians. J Clin Med. 2023; 12(7): 2714. doi: 10.3390/jcm12072714.
29. Dincel AS, Jørgensen NR. New emerging biomarkers for bone disease: sclerostin and dickkopf-1 (DKK1). Calcif Tissue Int. 2022; 112(2): 243–57.
30. Peacock M. Calcium metabolism in health and disease. Clin J Am Soc Nephrol. 2010; 5(Suppl 1): S23–30.
31. Carpenter TO, Imel EA, Holm IA, De Beur SMJ, Insogna KL. A clinician's guide to X-linked hypophosphatemia. J Bone Miner Res. 2011; 26(7): 1381–8.
32. Minisola S, Pepe J, Piemonte S, Cipriani C. The diagnosis and management of hypercalcaemia. BMJ. 2015; 350: h2723. doi: 10.1136/bmj.h2723.
33. Reddy P, Edwards LR. Magnesium supplementation in vitamin D deficiency. Am J Ther. 2017; 26(1): e124–32.
34. Group F the IIBMSW, Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, *et al.* Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: A need for international reference standards. Osteoporos Int. 2010; 22(2): 391–420.
35. Vasiliadis ES, Evangelopoulos DS, Kaspiris A, Benetos IS, Vlachos C, Pneumaticos SG. The role of sclerostin in bone diseases. J Clin Med. 2022; 11(3): 806. doi: 10.3390/jcm11030806.
36. Khan B, Nowson CA, Daly RM, English DR, Hodge AM, Giles GG, *et al.* Higher dietary calcium intakes are associated with

reduced risks of fractures, cardiovascular events, and mortality: A prospective cohort study of older men and women. J Bone Miner Res. 2015; 30(10): 1758–66.

37. Marroncini G, Martinelli S, Petrelli F, Bombardiere F, Sarnataro A, Martelli FS. Serum PTH ≥ 40 pg/ml as a marker of bone fragility and vitamin D deficiency in periodontitis patients: Biochemical, densitometric and genetic evidence. Biomolecules. 2025; 15(11): 1600. doi: 10.3390/biom15111600.
38. Holick MF. Vitamin D deficiency. N Engl J Med. 2007; 357(3): 266–81. doi: 10.1056/nejmra070553.
39. Khundmiri SJ, Murray RD, Lederer E. PTH and vitamin D. Compr Physiol. 2016; 6(2): 561–601.
40. Omran A, Atanasova D, Landgren F, Magnusson P. Sclerostin: From molecule to clinical biomarker. Int J Mol Sci. 2022; 23(9): 4751. doi: 10.3390/ijms23094751.