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Comparative evaluation of dual-energy X-ray absorptiometry (DEXA) and biochemical bone markers (P1NP, and Vitamin D3)

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Abstract

Background: Dual-energy X-ray absorptiometry (DEXA) remains the gold standard for evaluating bone mineral density (BMD), but its limitations in accessibility and cost have driven interest in biochemical markers like procollagen type I N-terminal propeptide (P1NP) and vitamin D3. This study aimed to assess the diagnostic alignment and predictive value of P1NP and vitamin D3 in relation to DEXA-based bone density classifications.

Methods: A cross-sectional comparative study was conducted on 150 adults in Basrah, Iraq, equally divided into three groups based on DEXA-derived T-scores: normal, osteopenia, and osteoporosis. Serum P1NP and vitamin D3 levels were measured using automated chemiluminescent immunoassays. Non-parametric statistical tests were used due to the skewed distribution of data. Kruskal-Wallis tests compared marker levels across groups, while Spearman's correlation assessed relationships with T- and Z-scores. Multiple linear regression evaluated their predictive capacity, and ROC analysis determined diagnostic accuracy.

Results: P1NP levels increased progressively across normal, osteopenic, and osteoporotic groups, with a statistically significant difference (p = 0.048). Vitamin D3 levels were lower in the osteoporosis group but did not significantly differ across all categories (p = 0.106). Correlation analysis revealed no significant relationship between P1NP and T- or Z-scores. Vitamin D3 showed a weak but significant positive correlation with T-score (r = 0.180, p = 0.027). In regression analysis, vitamin D3 significantly predicted T-scores (p = 0.002) but not Z-scores. ROC analysis showed poor discriminative ability for both markers, with AUCs ranging from 0.401 to 0.588.

Conclusion: While vitamin D3 demonstrated a modest correlation and predictive value for T-scores, neither P1NP nor vitamin D3 showed sufficient diagnostic power to substitute for DEXA. Their utility may lie in complementary roles, particularly in settings where imaging access is limited. Integration with imaging, rather than replacement, is recommended.

Keywords: DEXA, P1NP, Vitamin D3, bone mineral density, osteoporosis

Introduction

Bone mineral density (BMD) is a vital clinical marker of bone health, used to assess the risk of fragility fractures and identify metabolic bone disorders such as osteopenia and osteoporosis [1]. Osteoporosis, characterized by low BMD, leads to increased fracture risk and disability, especially in aging populations [2]. Dual-energy X-ray absorptiometry (DEXA) is considered the gold standard for BMD measurement due to its precision and reproducibility [3]. DEXA provides areal BMD by comparing bone mass to a standard reference, commonly at the spine or hip [4]. However, it has limitations, such as overestimating bone strength in larger bones and its limited availability in some healthcare settings [5]. Consequently, there is growing interest in alternative methods like quantitative ultrasonography and machine learning-based estimation from X-ray images to improve accessibility and cost-effectiveness [6, 7]. These tools can enhance screening and early intervention in resource-limited settings where DEXA may not be feasible.

Procollagen type I N-terminal propeptide (P1NP) is a sensitive biochemical marker of bone formation, reflecting collagen synthesis during bone remodeling, while vitamin D3 is essential for calcium absorption and mineral metabolism [8]. Supplementation with vitamin D3 significantly reduces P1NP and other turnover markers in postmenopausal women,

indicating decreased bone remodeling activity [9]. Similarly, vitamin D3 and calcium intake are correlated with lower P1NP and CTX-β levels, which are associated with higher bone mineral density (BMD) in osteopenic patients [10].

P1NP is negatively correlated with femoral neck BMD, highlighting its potential as a surrogate marker for osteopenia and osteoporosis risk [11]. However, high-dose vitamin D supplementation did not outperform standard-dose in improving BMD, suggesting limitations in its predictive value for structural bone changes [12]. Furthermore, studies show inconsistencies in the correlation between biochemical markers and imaging-based BMD scores, with some failing to show statistically significant associations [13]. Thus, despite strong biological rationale, the diagnostic utility of P1NP and vitamin D3 remains under refinement [14]. Further studies are needed to standardize cut-offs, validate predictive value, and align biochemical data with imaging tools.

The study was undertaken with the objective of evaluating the comparative diagnostic value of dual-energy X-ray absorptiometry and selected biochemical bone markers, specifically procollagen type I N-terminal propeptide (P1NP) and vitamin D3, in the detection of low bone mineral density. It was designed to determine whether these biochemical measures of bone formation and mineral metabolism could provide meaningful diagnostic alignment with, or potential supplementation to, the gold standard imaging method. The intention was to assess how well P1NP and vitamin D3 reflected bone status across the spectrum of normal density, osteopenia, and osteoporosis.

A further objective of the research was to establish whether the levels of these markers varied in a statistically significant way among the three diagnostic categories. The investigation also sought to clarify correlations between P1NP and vitamin D3 concentrations with the T-scores and Z-scores generated by DEXA scanning. Through this approach, the study aimed to identify patterns of association that could indicate whether the biochemical markers were sensitive to bone density changes measured by imaging.

The analysis was additionally directed toward testing the predictive capacity of these markers through regression models and evaluating their ability to discriminate between normal bone density and cases of osteopenia or osteoporosis using ROC curve analysis. By doing so, the study sought to determine if P1NP and vitamin D3 held sufficient diagnostic performance to be considered as practical adjuncts or alternatives to imaging in selected clinical contexts. Ultimately, the work was carried out to clarify whether integrating biochemical assessment with densitometry could enhance early detection and improve the diagnostic framework for low bone mineral density.

Methodology

Study Design and Setting: The study was conducted as a cross-sectional comparative analysis that examined bone mineral density measurements alongside biochemical indicators of bone formation and mineral metabolism. It was carried out within a fixed timeframe in Basrah, Iraq, where patients routinely referred for bone health evaluation were enrolled. The design enabled the acquisition of data from untreated individuals at a single point in time, ensuring that both imaging and biochemical findings reflected natural physiological states rather than treatment effects. Recruitment and diagnostic processes took place in government teaching hospitals that functioned as primary referral centers, while the laboratory component of the study was performed in a specialized private diagnostic facility equipped with advanced immunoassay systems. This dual-site arrangement ensured access to both the clinical population of interest and the technical resources necessary for high-quality biochemical testing. The integration of diverse healthcare settings also reflected real clinical pathways, enhancing the generalizability of the findings. The structure of the design emphasized comparability across groups with different bone density classifications and provided a framework to evaluate the diagnostic performance of biochemical markers in relation to the imaging gold standard.

Study Population and Referral Pathways

The study population was formed of adults, both sexes, who were first referred to a diagnostic assessment of bone health. Subjects were recruited mainly from ambulatory clinics in Basrah, and were generally representative of both urban and semi-urban areas in the region. Diabetic patients were referred by specialists in rheumatology and orthopedics, and spine surgery by referring physicians in each speciality who saw patients presenting symptoms or clinical signs indicating reduced bone strength. Chronic musculoskeletal pain, postural modification, or increased propensity to low trauma fractures were present in many cases. The referral process was carried out according to usual hospital practice and a decision on need for further work up was taken by physicians independent of the study. The patients who were referred in such a fashion were then scheduled for densitometric scanning and associated laboratory study. The recruitment of participants reflected routine clinical practice and thus the sample did indeed represent real-life practice. Women were, as might be expected in the epidemiology of low bone mineral density, predominately postmenopausal and men were also recruited if their clinical presentations merited further investigation. This indigenous referral system lent credibility and external validity to the study population.

Eligibility Criteria: Potential participants were identified based on pre-defined criteria in order to maintain internal consistency of imaging and biochemical values. Only adults (skeletally mature) were included as this assured that evidence pertaining to bone metabolism was representative of stable physiological events, rather than developmental growth. All patients were included if they had not previously received a measurement of bone products or biochemical marker analysis which influenced their medical management or life-style changes due to the results of prior testing. Patients were excluded if they were under the influence of pharmacological agents that would interfere with the normal range of biochemical indices as they related to bone metabolism. Tubero-infectious diseases and socioeconomic conditions causing altered bone turnover (systemic diseases of bone remodeling, like chronic hepatic or renal dysfunction, malignancy or unregulated endocrine diseases as well as)

Were excluded recent hospital admission, major surgery or severe immobilization were other reasons not to include these individuals, as these events are associated with transient but detectable changes in skeletal metabolism. Pregnant and lactating women were excluded because

plasma calcium and vitamin D homeostasis are physiologically altered in these states. Adults who were physically or cognitively impaired to the extent that they could not provide an informed consent to participate in Known Minor Risks A.R. exercise testing for diagnosis of IPA or to cooperate with study procedures were also excluded.

Sampling Method and Group Allocation

The sampling protocol was a purposive stratified sample, conducted so that once data for a patient was available the study group was split between the diagnostic categories of bone density evenly. The design called for fifty subjects to be tested in each of the three groups to be defined as normal bone density, osteopenia or osteoporosis, for a total of one hundred and fifty participants. Subjects were assigned solely on the basis of T-scores from dual-energy X-ray absorptiometry and were classified into an appropriate category as soon as results were generated. Once the predefined number for a category had been reached, no further members could be recruited for that research group, thus allowing for an even distribution across the bone health spectrum. This made it possible to compare biochemical markers between groups of constant size and thereby strengthening the statistical analyses. The method also reduced the potential for skewing that might have occurred from a post hoc analysis if one diagnostic category was over-represented. The study was strengthened by enforcing symmetry among sample sizes, aligning thresholds for the developmental basis of progressing from normal bone density to advanced end-stage mineral loss-and allowed for meaningful differences to be detected in the P1NP and vitamin D3 values. The architecture of the disease offered a strong ground for testing the diagnostic utility of these markers with respect to imaging.

DEXA Scan Procedure and Diagnostic Categorization

Bone mineral density was measured using the DEXA STRATOS bone densitometer manufactured by DMS Imaging (Diagnostic Medical Systems Group, France). This instrument was dedicated to assessing bone mass at clinically validated anatomical sites, specifically the lumbar spine (L1-L4) and the femoral neck, which are the most predictive of fracture risk. Each scan was performed by radiologic technologists trained in standardized positioning techniques to reduce operator variability and prevent motion artifacts. The device software automatically calculated T-scores and Z-scores using reference data stratified by sex, age, and ethnicity. For diagnostic allocation, the T-score was the sole criterion applied in accordance with World Health Organization thresholds: values of −1.0 or higher indicated normal bone density, those between −1.0 and −2.5 signified osteopenia, and values equal to or less than −2.5 were diagnostic of osteoporosis. Z-scores were recorded for contextual information but were not used in group assignment. All scans were scheduled in the morning hours to coincide with biochemical testing, thereby reducing variability related to circadian influences. Routine phantom calibration was carried out daily on the STRATOS densitometer to maintain precision. The results were archived electronically, providing quantitative values and graphical outputs for use in subsequent analysis and diagnostic categorization.

Biochemical Measurement of Serum P1NP

Serum concentrations of procollagen type I N-terminal propeptide (P1NP) were determined using the MAGLUMI® P1NP CLIA kit, catalog number 130219017M, supplied by Snibe Diagnostics. Analyses were conducted on the MAGLUMI® 1000 automated chemiluminescent immunoassay analyzer, a fully automated platform designed for high-precision biomarker testing. The assay was based on a two-site sandwich immunoassay principle, in which two monoclonal antibodies targeted distinct epitopes on the P1NP molecule. Magnetic particles coated with a capture antibody bound to the analyte, while an acridinium ester-labeled detection antibody generated a chemiluminescent signal directly proportional to the serum concentration. The analytical range of the assay was 5-1200 ng/mL, with a limit of detection of 5 ng/mL. The intra-assay coefficient of variation was maintained at less than 5.0%, and the inter-assay coefficient of variation was below 7.0%, ensuring reproducibility across runs. Each batch included manufacturer-provided calibrators and quality control reagents, with calibration performed at five concentration points to ensure accuracy. Assay runtime was approximately 40 minutes, and results were expressed in ng/mL. All measurements were performed in duplicate, and final values were averaged to reduce variability. Laboratory staff conducting the analysis were blinded to participants' bone density status to maintain objectivity.

Biochemical Measurement of Serum Vitamin D3

Serum levels of 25-hydroxyvitamin D3 [25(OH)D3] were measured using the MAGLUMI® 25-OH Vitamin D CLIA kit, catalog number 130219016M, manufactured by Snibe Diagnostics. All analyses were carried out on the MAGLUMI® 1000 automated chemiluminescent immunoassay analyzer, which allowed precise and reproducible quantification. The assay operated on a competitive binding principle in which serum 25(OH)D3 competed with a labeled analog for a finite number of antibody binding sites coated onto magnetic microbeads. Following incubation, unbound material was removed by washing, and the bound fraction was detected through a chemiluminescent reaction initiated by acridinium ester labels. The intensity of emitted light was inversely proportional to the concentration of vitamin D3 in the sample. The analytical measurement range extended from 4.0 to 120.0 ng/mL, with a limit of detection of 1.5 ng/mL. The intra-assay coefficient of variation was less than 6.5%, while the inter-assay coefficient of variation was maintained below 8.5%, confirming consistent assay performance. Each test run incorporated manufacturer-supplied calibrators and low and high concentration controls to validate accuracy. Results were expressed in ng/mL, and the total processing time was approximately 40 minutes. Laboratory personnel responsible for analysis were blinded to clinical classifications to avoid interpretive bias.

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Data were initially tested for normality using the Shapiro-Wilk test, and since most variables did not follow a normal distribution, non-parametric methods were employed for group comparisons. Descriptive statistics for serum P1NP and vitamin D3 levels were presented as medians with interquartile ranges (IQR).

To compare these biochemical markers across the three bone density categories normal, osteopenia, and osteoporosis a Kruskal-Wallis H test was used. A p-value < 0.05 was considered statistically significant.

Associations between biochemical marker levels and DEXA-derived T-scores and Z-scores were assessed using Spearman's rank-order correlation, due to non-parametric data distribution. Results were reported with correlation coefficients (r) and corresponding p-values.

To evaluate the predictive capability of P1NP and vitamin D3 for bone density scores, multiple linear regression models were constructed separately for T-scores and Z-scores. Standardized beta coefficients, 95% confidence intervals, and p-values were reported to determine statistical significance.

Diagnostic performance was further assessed using Receiver Operating Characteristic (ROC) curve analyses, comparing each biochemical marker's ability to distinguish between:

- Normal vs. Osteopenia
- Normal vs. Osteoporosis

The Area under the Curve (AUC) and p-values were calculated to evaluate the markers' discriminative power. An AUC of 0.5 was interpreted as no discrimination, whereas values closer to 1.0 indicated better diagnostic performance. All tests were two-tailed, and significance was predefined at $p<0.05$.

Results

Table R2-1: Descriptive statistics of P1NP and Vitamin D3 across DEXA categories

Variable	Normal (N=50)	Osteopenia (N=50)	Osteoporosis (N=50)	P value
P1NP (ng/mL) Median (IQR)	43.550 (35.921-90.752)[a]	48.565 (30.325-77.680)[b]	69.150 (37.600-111.250)[c]	0.048***
Vitamin D3 (ng/mL) Median (IQR)	15.45 (10.60-22.50)[a]	15.40 (10.75-22.70)[a]	12.85 (9.10-17.11)[b]	0.106***

Data presented as median (interquartile range). ***Kruskal-Wallis test. Predetermined significance set at p<0.05.*

The comparison of biochemical markers across diagnostic groups revealed a gradual rise in P1NP values from normal to osteopenic and osteoporotic patients, with the difference reaching statistical significance. This trend indicated increased bone formation activity in individuals with reduced bone mineral density, likely reflecting a compensatory response to ongoing bone loss. Vitamin D3 levels were lowest in the osteoporotic group, significantly lower than both normal and osteopenic groups, although no difference was found between the latter two. These findings highlighted a pattern in which reduced vitamin D3 availability coincided with advanced mineral loss, while P1NP increased progressively with disease severity.

Table R2-2: Correlation of P1NP and Vitamin D3 with DEXA scores

Biomarker	Correlation with T-score (r)	p-value	Correlation with Z-score (r)	p-value
P1NP	−0.155	0.161	−0.350	0.673
Vitamin D3	0.180	0.027	0.136	0.097

Spearman's rank correlation coefficient. Predetermined significance set at $p<0.05$.

Correlation analysis demonstrated that P1NP was not significantly associated with either T-scores or Z-scores, suggesting limited utility of this marker in reflecting densitometric status in this cohort. Vitamin D3, however, showed a weak positive correlation with T-scores, indicating that higher vitamin D3 levels tended to accompany better bone density outcomes. Although the correlation with Z-scores did not reach statistical significance, the overall pattern suggested that vitamin D3 may play a supportive but not definitive role in bone density classification. These results pointed to the limited diagnostic alignment of P1NP and a modest contributory value for vitamin D3.

Table R2-3: Multiple linear regression predicting Z-score (P1NP and Vitamin D3 only)

Predictor	B	Std. Error	Beta	p-value	95% CI (Lower-Upper)
P1NP	−0.001	0.002	−0.057	0.481	−0.005 - 0.003
Vitamin D3	0.017	0.010	0.134	0.090	−0.003 - 0.036

Multiple linear regression model. Predetermined significance set at $p<0.05$.

In the regression model predicting Z-scores, neither P1NP nor vitamin D3 emerged as strong independent predictors. The coefficient for P1NP was small, negative, and statistically non-significant, indicating no consistent effect on Z-score variation. Vitamin D3 displayed a positive coefficient, implying a potential association with higher Z-scores, but this relationship failed to achieve statistical significance at the predetermined threshold. The wide confidence intervals surrounding both predictors reinforced the limited explanatory power of these biochemical measures when evaluated against densitometric indices. These findings underscored the restricted capacity of P1NP and vitamin D3 to account for variability in Z-scores.

Table R2-4: Multiple linear regression predicting T-score (P1NP and Vitamin D3 only)

Predictor	B	Std. Error	Beta	p-value	95% CI (Lower-Upper)
P1NP	−0.001	0.002	−0.024	0.749	−0.005 - 0.004
Vitamin D3	0.033	0.011	0.222	0.002	0.012 - 0.054

Multiple linear regression model. Predetermined significance set at $p<0.05$.

The regression model for predicting T-scores produced a different outcome compared with the Z-score model. P1NP again demonstrated no significant contribution, with its coefficient remaining close to zero. In contrast, vitamin D3 showed a statistically significant positive effect, with each unit increase in vitamin D3 corresponding to an increase in T-score. This suggested that vitamin D3 status was directly linked to better bone density as classified by T-scores. The standardized beta coefficient further highlighted vitamin D3's relative importance among the predictors. These results provided evidence that vitamin D3 may have some diagnostic value in relation to T-score classification.

Table R2-5: ROC analysis for distinguishing Normal vs Osteopenia (P1NP and Vitamin D3)

Biomarker	AUC	p-value
P1NP	0.448	0.374
Vitamin D3	0.515	0.058

Receiver Operating Characteristic (ROC) curve analysis. Predetermined significance set at $p<0.05$.

Table R2-5
The ROC analysis of P1NP and vitamin D3 for distinguishing normal bone density from osteopenia revealed poor diagnostic performance. P1NP produced an AUC of 0.448, which was below the threshold of random classification, while vitamin D3 yielded an AUC of 0.515, only marginally above chance level. Neither biomarker reached statistical significance, indicating that they could not reliably separate normal individuals from those with osteopenia. These results suggested that, despite observed trends in descriptive statistics, neither P1NP nor vitamin D3 demonstrated meaningful discriminative power for early stages of bone loss when tested against the gold-standard DEXA classification

Table R2-6: ROC analysis for distinguishing Normal vs Osteoporosis (P1NP and Vitamin D3)

`Biomarker	AUC	p-value
P1NP	0.588	0.128
Vitamin D3	0.401	0.057

Receiver Operating Characteristic (ROC) curve analysis. Predetermined significance set at $p<0.05$.

On the other hand, when the test is conducted for the ability to differentiate normal bone density or osteoporosis, then again, PNP and vitamin D3 had limited diagnostic ability. P1NP showed moderate discrimination with an AUC (area under curve) of 0.588, whereas vitamin D3 showed poor discrimination with an AUC of 0.401, which is below chance. Neither marker was statistically significant, which confirmed that each was insufficient to stand alone for imaging in identifying advanced bone loss. These data confirmed that, while both markers could yield suggestive information on bone metabolism, they had not the sensitivity and specificity to be used alone as diagnostic tools for osteoporosis, and once more showed the overwhelming importance of DEXA in the clinical examination.

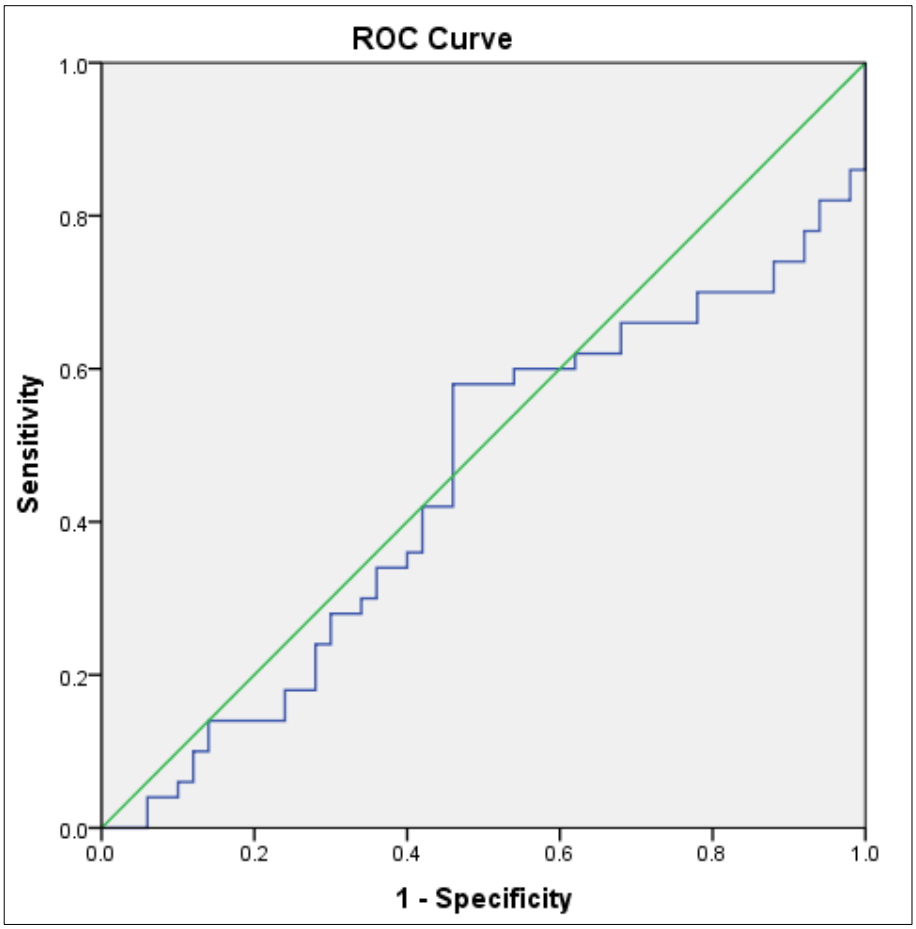

Fig 1: ROC curve for classifying osteopenia by P1NP

Compared with normal bone density for distinguishing osteopenia, the diagnostic performance of P1NP in the ROC curve was very poor. Their curve will be close to their diagonal reference line and there was not a great ability to discriminate between groups. The area under the curve was 0.448 which is below what would be expected for random classification and the p value confirmed that there was not statistical significance. This was an interesting observation suggesting that serum P1NP is not measurably different between patients with early bone loss compared to patients with normal bone density, which reflects its inadequacy as a stand-alone diagnostic tool for osteopenia, compared to the current gold standard DEXA scan.

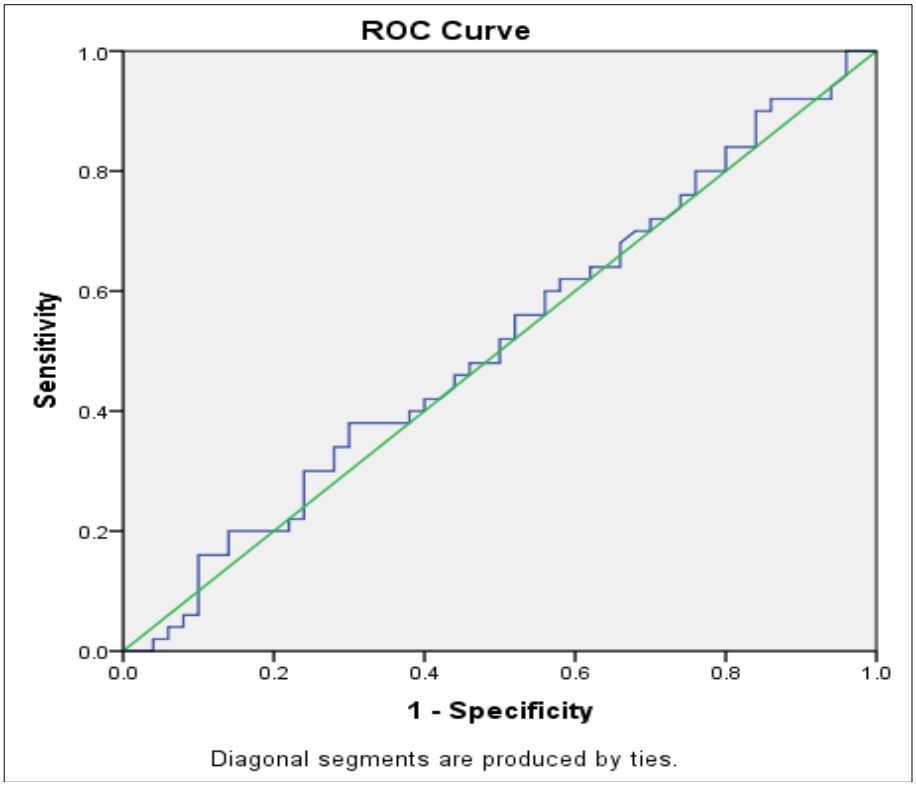

Fig 2: ROC curve for classifying osteopenia by Vitamin D

The ROC curve in osteopenia detection for Vitamin D compared with normal bone density also exhibited poor diagnostic accuracy. The curve was very close to the chance diagonal resulting in an area under the curve of 0.515, which only slightly exceeds the chance classification. In individuals with osteopenia, there was a trend toward lower levels, but statistical significance was not achieved, suggesting that a vitamin D level would be unreliable at differentiating between normal and osteopenic subjects. This implied that whilst vitamin D deficiency was more apparent in later stages of bone loss, it was not sensitive or specific enough for earlier stages. All things considered, assessment of vitamin D was inadequate alone in identifying osteopenia in the absence of corroborating imaging information.

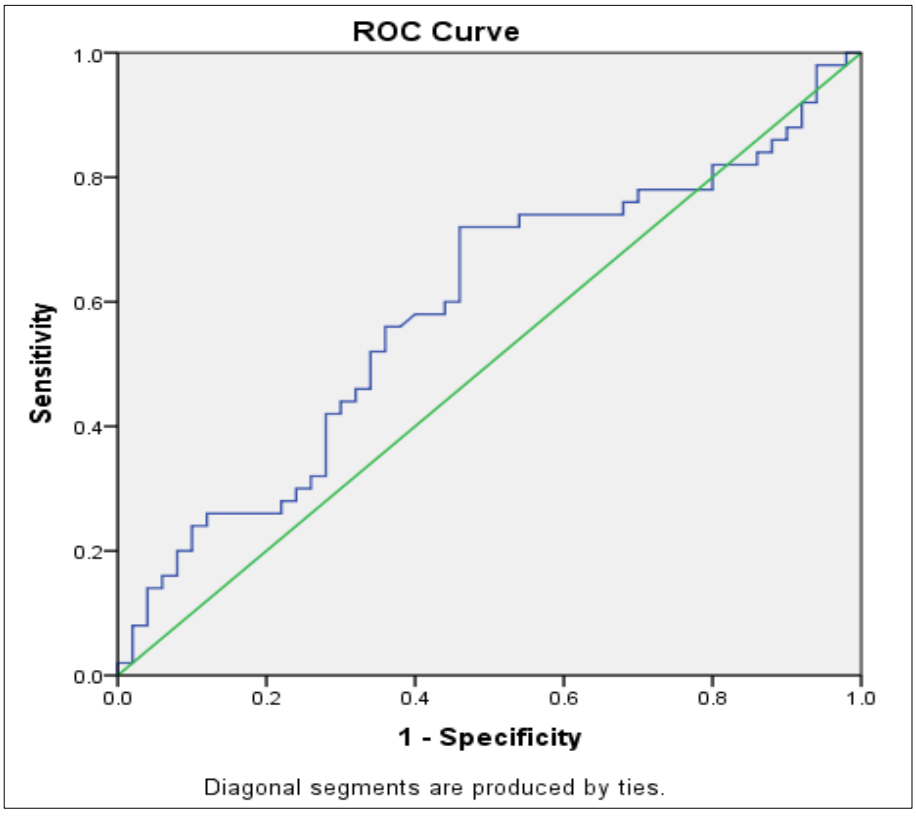

Fig 3: ROC curve for classifying osteoporosis by P1NP

The ROC curve evaluating P1NP for distinguishing osteoporosis from normal bone density demonstrated slightly improved but still inadequate diagnostic capability. The area under the curve reached 0.588, representing poor accuracy, and the result did not achieve statistical significance. Although median P1NP levels were higher in osteoporotic patients, the overlap with other groups reduced its value as a discriminatory marker. The ROC curve remained relatively close to the line of no discrimination, confirming that P1NP could not be considered a reliable tool for osteoporosis diagnosis. This figure highlighted the weak predictive contribution of P1NP in advanced bone mineral loss.

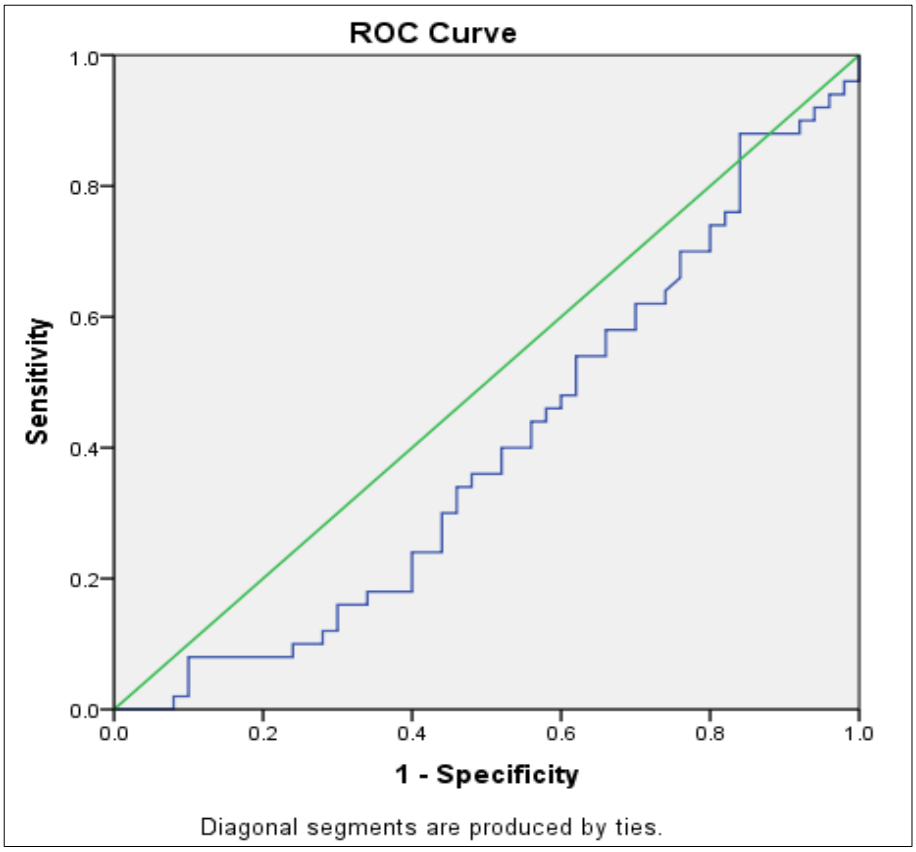

Fig 4: ROC curve for classifying osteoporosis by Vitamin D

The ROC curve for vitamin D in distinguishing osteoporosis from normal bone density revealed no diagnostic utility. The curve lay below the reference diagonal, with an AUC of 0.401, suggesting worse than random performance. This result, coupled with a non-significant p-value, confirmed that vitamin D measurements could not be used as a diagnostic test for osteoporosis. Although vitamin D deficiency was more prevalent in patients with advanced bone loss, the overlap with non-osteoporotic groups prevented effective classification. This figure demonstrated that vitamin D lacked both sensitivity and specificity, reaffirming that DEXA scanning remained the gold standard diagnostic tool.

Discussion

In the present study, the observed elevation in P1NP levels across the progression from normal bone density to osteopenia and osteoporosis indicates a possible compensatory increase in bone formation activity in response to ongoing bone resorption. The significant difference in P1NP values ($p = 0.048$) supports this interpretation. Clinically, this pattern may reflect an attempt by the skeletal system to counteract mineral loss through remodeling processes, although such compensatory activity may be insufficient to restore bone mass. Concurrently, vitamin D3 levels were lower in the osteoporotic group compared to the normal and osteopenic groups, although the overall difference did not reach statistical significance ($p = 0.106$). This reduction in vitamin D3 in more severe bone loss aligns with known roles of vitamin D in calcium absorption and bone health, suggesting that deficiency may exacerbate bone deterioration.

These findings are supported by several recent studies. For instance, Zhang *et al.* found that patients with lower baseline vitamin D levels exhibited higher P1NP concentrations, and supplementation with vitamin D led to significant reductions in P1NP, implying that low vitamin D levels are associated with increased bone turnover [15]. This supports the present study's findings on the inverse relationship between vitamin D and P1NP.

Contrasting evidence is provided by Masik *et al.*, who reported that P1NP levels decrease with age and disease severity in patients with chronic obstructive pulmonary disease (COPD), suggesting suppressed bone formation in advanced disease [16]. This finding challenges the present study's indication of increased P1NP in osteoporosis, possibly due to differing pathophysiological mechanisms between osteoporosis and COPD-related bone loss.

Further support for the present study's trend is found in Feng *et al.*, who observed elevated P1NP in osteoporotic patients with fractures, along with decreased vitamin D levels. Their study concluded that both P1NP and vitamin D

are valuable in predicting fracture risk, reinforcing the current study's clinical interpretation that high P1NP and low vitamin D3 are markers of active and advanced bone turnover [17].

Conversely, a study by Miśkiewicz-Orczyk *et al.* did not find a statistically significant relationship between vitamin D3 levels and bone mineral density in patients with idiopathic benign paroxysmal positional vertigo (BPPV), challenging the clinical relevance of vitamin D3 as a diagnostic marker for bone loss [18]. This discrepancy may stem from differences in disease mechanisms or population characteristics, highlighting the need to contextualize findings within specific clinical settings.

Moreover, Pronina *et al.* documented that vitamin D deficiency was associated with higher P1NP and lower bone mineral density in pediatric patients with congenital epidermolysis bullosa, aligning with the present study's pattern [19]. This further supports the link between vitamin D status and bone turnover activity as reflected by P1NP, even outside the adult osteoporotic population.

In summary, the present study's findings are largely consistent with recent literature demonstrating that vitamin D deficiency is common in low bone density states and is often accompanied by elevated P1NP, reflecting active bone remodeling. Discrepancies observed in other studies may relate to differences in underlying disease mechanisms, age groups, or population characteristics. Nonetheless, the overall trend supports the notion that both P1NP and vitamin D3 serve as valuable, albeit limited, adjuncts to imaging in understanding the metabolic profile of bone health.

The present study explored the correlation between serum levels of procollagen type I N-terminal propeptide (P1NP) and vitamin D3 with DEXA-derived T-scores and Z-scores, representing bone mineral density. The results showed no statistically significant correlation between P1NP and either T-score ($r = -0.155$, $p = 0.161$) or Z-score ($r = -0.350$, $p = 0.673$), suggesting limited utility of P1NP as a biochemical marker of bone density in the sampled population. In contrast, vitamin D3 showed a weak but statistically significant positive correlation with T-scores ($r = 0.180$, $p = 0.027$), indicating that individuals with higher vitamin D3 levels had slightly better bone density outcomes. However, the correlation with Z-scores was not significant ($r = 0.136$, $p = 0.097$), pointing to a modest and somewhat inconsistent role of vitamin D3 in bone density classification.

These findings align partially with several recent studies that also investigated these biomarkers. For instance, Elghaiaty *et al.* found a strong and statistically significant positive correlation between vitamin D3 levels and DEXA Z-scores in children with hemophilia A, with $r = 0.693$ and $p<0.001$ [20]. This reinforces the present study's conclusion regarding the beneficial link between vitamin D and bone density, although the correlation strength in their pediatric cohort was notably stronger [20].

Similarly, a 2024 study by Hanna *et al.* reported that monthly high-dose vitamin D3 supplementation significantly improved DEXA Z-scores in children and adolescents with sickle cell disease. This intervention led to a measurable improvement in bone mineral density, further supporting the present study's findings on the contributory role of vitamin D3, especially in deficient populations [21].

In contrast, Battaglia *et al.* conducted a study on long-term kidney transplant recipients and found that while vitamin D supplementation increased serum 25(OH)D levels, it did not result in statistically significant improvements in T-scores or Z-scores, particularly at the femoral neck [22]. This finding directly opposes the modest correlation found in the present study, suggesting that the relationship between vitamin D and bone density may be population-specific and influenced by underlying health conditions.

Further disagreement arises from the findings of Sinha and Garg, who reported no significant correlation between vitamin D levels and T-scores in a sample of apparently healthy Indian adults [23]. Despite the widespread assumption that vitamin D supports bone health, this study concluded that vitamin D levels alone were insufficient as a standalone predictor for bone density, mirroring the ambiguity seen in the Z-score results of the present study.

Soliman *et al.* added further evidence of the relationship between vitamin D and bone mineral density by studying patients with juvenile idiopathic arthritis (JIA). They found that DEXA Z-scores were significantly lower in vitamin D-deficient patients, echoing the weak but present association observed in the current study [24].

In regard to P1NP, there was a lack of strong recent literature showing consistent correlations with DEXA-derived T- or Z-scores in broader clinical contexts, which supports the present study's conclusion. However, Chen *et al.* conducted a multicenter study of adults with osteoporosis and found that P1NP levels were inversely associated with bone mineral density, and positively correlated with bone turnover and fracture risk, suggesting that P1NP might be more useful as a bone turnover marker rather than a direct correlate of bone density scores [25].

These comparisons highlight the variability in the diagnostic performance of vitamin D3 and P1NP across different patient groups and research designs. While vitamin D shows modest and population-dependent correlations with bone density, P1NP appears more relevant to bone turnover dynamics than to direct bone mineral content, especially in cross-sectional assessments. The present study confirms that these biomarkers cannot replace DEXA but may serve as supplemental tools in select clinical scenarios.

The current study revealed contrasting outcomes in the predictive value of vitamin D3 and P1NP in relation to T-scores and Z-scores derived from DEXA. When examining Z-scores, neither vitamin D3 nor P1NP emerged as statistically significant independent predictors, as evidenced by p-values of 0.090 and 0.481, respectively. This suggests a weak or inconsistent association between these biomarkers and bone density measurements standardized to age-matched peers. However, when evaluating T-scores which compare bone mineral density to a young, healthy reference population vitamin D3 showed a significant positive correlation ($p = 0.002$), indicating that higher vitamin D3 levels are associated with improved bone density. In contrast, P1NP continued to demonstrate no meaningful contribution to T-score prediction ($p = 0.749$). Clinically, this highlights that vitamin D3 may have some diagnostic value for overall bone health relative to normative values, but not in age-matched contexts. P1NP, as a marker of bone formation, appears less useful in directly reflecting bone mass as measured by densitometry in this cross-sectional analysis. These findings affirm the current view that vitamin D status contributes to bone density but must be interpreted cautiously, particularly when attempting to draw

conclusions about age-standardized indices such as Z-scores.

Several studies support the present study's finding that vitamin D3 is positively associated with improved T-scores. For instance, in a Korean adolescent population, higher serum 25(OH)D levels were significantly associated with better BMD Z-scores across lumbar spine, femur neck, and whole body, even after adjusting for confounding factors such as BMI and physical activity [26]. This aligns with the observed T-score relationship in the current study and reinforces the role of vitamin D in bone mass accrual during growth periods [26]. Similarly, in a large pediatric hemophilia cohort, vitamin D3 was strongly correlated with Z-scores, suggesting that sufficient vitamin D levels may contribute to healthier bone mineral profiles in at-risk populations [20].

Furthermore, a study of children and youth with type 1 diabetes found that vitamin D3 supplementation (both dairy and pharmacological forms) significantly improved T- and Z-scores after a year-long intervention, particularly among females, highlighting the value of vitamin D in promoting bone density during critical developmental stages [27]. A separate trial among Iraqi postmenopausal women also found that vitamin D deficiency was associated with significantly lower T-scores, consistent with the present study's outcomes for T-score prediction [28].

On the contrary, several studies diverge from the present findings. Notably, in long-term kidney transplant recipients, Battaglia *et al.* reported that although vitamin D supplementation improved serum 25(OH)D levels, it failed to significantly change T- or Z-scores, particularly at femoral sites [29-31]. These studies suggest that while vitamin D sufficiency is essential, its isolated supplementation may not translate into measurable improvements in BMD in certain populations.

Another dissenting result comes from Divani *et al.*, where cholecalciferol supplementation in vitamin D-deficient hemodialysis patients did not improve T- or Z-scores or lumbar spine BMD, although vitamin D levels significantly increased [32]. This reinforces the idea that the benefits of vitamin D on bone density might be population-specific and influenced by comorbid conditions such as chronic kidney disease.

Moreover, a study by Kalpana *et al.* on national-level athletes revealed no significant correlation between serum vitamin D3 levels and T- or Z-scores, although a notable association was found between BMD scores and performance measures like speed [33]. This suggests that functional bone strength may not always parallel vitamin D levels, particularly in physically active populations.

Additional studies such as those by Han *et al.* and Hammoud *et al.* support a positive relationship between vitamin D deficiency and lower BMD or T-scores in gestational diabetes and adolescent scoliosis patients, respectively, further validating the present study's finding on the significance of vitamin D in T-score prediction [34, 35].

In contrast, Mat Ali *et al.* found no significant difference in BMD Z-scores between thyroid disease patients and healthy controls, despite variances in vitamin D and bone marker levels. This challenges the universality of vitamin D's effect on Z-scores and aligns with the present study's findings regarding vitamin D's limited predictive capacity for Z-scores [36].

Taken together, these comparisons suggest that while vitamin D3 has some value as a predictor of bone density via T-scores, its impact on Z-scores is modest and highly context-dependent. The conflicting evidence across populations pediatric, postmenopausal, renal, or athletic points to the need for individualized interpretation of vitamin D's diagnostic utility. P1NP's lack of correlation in the present study is similarly echoed in the broader literature, suggesting its stronger relevance in bone turnover or remodeling processes rather than static BMD values derived from imaging. Therefore, integrating biochemical markers with imaging should remain a supplementary, not substitute, approach in osteoporosis diagnostics.

Conclusion

This study comprehensively evaluated the diagnostic performance of serum P1NP and vitamin D3 levels against DEXA-derived bone mineral density indices among untreated adults. The findings revealed that while P1NP levels significantly increased across the spectrum from normal bone density to osteoporosis, the marker lacked meaningful correlation with DEXA T- and Z-scores and failed to demonstrate predictive capacity in regression models. Conversely, vitamin D3 exhibited a statistically significant, albeit weak, correlation with T-scores and emerged as a significant predictor in the corresponding regression model, suggesting some utility in evaluating general bone health.

However, both markers demonstrated poor discriminative power in ROC curve analyses, with AUCs close to or below 0.5, reinforcing their inadequacy as standalone diagnostic tools. P1NP and vitamin D3 could not reliably distinguish between normal, osteopenic, or osteoporotic states, particularly in early stages of bone loss. These findings confirm that despite the biological plausibility of using bone turnover and metabolic markers to infer structural bone changes, their clinical utility as diagnostic alternatives remains limited.

In practice, vitamin D3 may serve as a supportive marker in conjunction with densitometric findings, especially in cases where imaging is inaccessible or as part of broader metabolic profiling. P1NP, more reflective of bone remodeling activity than bone mass itself, may hold potential value in monitoring treatment responses or fracture risk but not in baseline diagnostic classification.

The results underscore the continued primacy of DEXA scanning in osteoporosis diagnostics and highlight the necessity of multimodal approaches when integrating biochemical assessments. Future research should aim to identify novel biomarker combinations, establish standardized thresholds, and validate these tools across diverse populations to improve non-invasive diagnostic accuracy for bone health.

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