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Correlations of Serum Pentosidine with other Biochemical Parameters in Iraqi Individuals with Osteopenia and Osteoporosis

Mawj. S. Ibrahim *, Namir I. A. Haddad
Department of chemistry, College of Sciences, University of Baghdad, Baghdad, Iraq

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Abstract

Osteoporosis (OS) and osteopenia (OP) are systemic skeletal conditions characterized by decreased bone mass and deterioration of bone microarchitecture, leading to heightened fragility and an increased risk of fractures. Pentosidine (PTD), an advanced glycation end-product formed through the cross-linking of norleucine and ornithine residues, serves both as a biochemical marker and a cross-linking agent that plays a role in bone remodeling. This study aims to assess serum PTD levels in Iraqi patients with OP and OS and explore their correlation with various biochemical parameters. The study involved 121 participants divided into three groups: 42 with OS, 37 with OP, and 42 healthy controls. Blood samples were analyzed for PTD, vitamin D3, calcium, magnesium, phosphorus, and lipid profile levels. PTD and vitamin D3 levels were determined using enzyme-linked immunosorbent assay (ELISA). The findings showed significantly higher PTD levels in both OP and OS groups compared to the control group, which correlated with reduced bone mineral density (BMD). Additionally, patients in the OP and OS groups exhibited lower levels of calcium and vitamin D3, while magnesium and phosphorus levels showed no statistically significant differences. Lipid profile analysis indicated higher levels of triglycerides (TG), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) in the OS group ($p < 0.001$). Receiver operating characteristic (ROC) curve analysis demonstrated a high diagnostic accuracy of PTD in detecting OP and OS, with an area under the curve (AUC) of 1.000. These findings suggest that PTD may serve as a reliable and sensitive biomarker for the early detection and assessment of osteopenia and osteoporosis.

Keywords: Osteoporosis, Osteopenia, PTD

ارتباطات البنتوسيدين في المصل مع المعايير الكيميائية الحيوية الأخرى لدى الأفراد العراقيين المصابين بلين العظام وهشاشة العظام

موج سعدون ابراهيم *, نمير ابراهيم عباس
قسم كيمياء, كلية العلوم, جامعة بغداد, بغداد, العراق

الخلاصة

هشاشة العظام (OS) ولين العظام (OP)هما حالتان هيكليتان جهازيتان تتميزان بانخفاض كتلة العظام

*Email: mawj.saadoun2305@sc.uobaghdad.edu.iq

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وتدهور بنيتها الدقيقة، مما يؤدي إلى زيادة هشاشتها وزيادة خطر الإصابة بالكسور. يعمل البنتوسيدين (PTD)، وهو منتج نهائي متقدم للجليكوزيل يتكون من خلال الارتباط المتبادل لبقايا النوروليوسين والأورنيثين، كعلامة بيوكيميائية وعامل ارتباط متبادل يلعب دورًا في إعادة تشكيل العظام. تهدف هذه الدراسة إلى تقييم مستويات PTD في مصل المرضى العراقيين المصابين بهشاشة العظام وهشاشة العظام واستكشاف ارتباطها بمختلف المعايير البيوكيميائية. شملت الدراسة 121 مشاركًا مقسمين إلى ثلاث مجموعات: 42 مصابًا بهشاشة العظام، و37 مصابًا بهشاشة العظام، و42 من الأصحاء. تم تحليل عينات الدم لمستويات PTD وفيتامين D3 والكالسيوم والمغنيسيوم والفوسفور ومستوى الدهون. تم تحديد مستويات PTD وفيتامين D3 باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA). أظهرت النتائج ارتفاعًا ملحوظًا في مستويات PTD في كلٍّ من مجموعتي OP وOS مقارنةً بالمجموعة الضابطة، مما ارتبط بانخفاض كثافة المعادن في العظام (BMD). بالإضافة إلى ذلك، أظهر مرضى مجموعتي OP وOS مستويات أقل من الكالسيوم وفيتامين D3، بينما لم تُظهر مستويات المغنيسيوم والفوسفور أي فروق ذات دلالة إحصائية. أشار تحليل ملف الدهون إلى ارتفاع مستويات الدهون الثلاثية(TG)، والبروتين الدهني منخفض الكثافة (LDL)، والبروتين الدهني منخفض الكثافة جدًا (VLDL)في مجموعة البقاء العظمي ($P < 0.001$).. وأظهر تحليل منحنى خاصية تشغيل المُستقبِل (ROC) دقة تشخيصية عالية لفحص PTD في الكشف عن OP وOS، حيث بلغت المساحة تحت المنحنى 1.000 (AUC). وتشير هذه النتائج إلى أن فحص PTD قد يكون بمثابة مؤشر حيوي موثوق وحساس للكشف المبكر عن هشاشة العظام وهشاشتها وتقييمهما.

Introduction

Osteoporosis is a progressive disease characterized by weakened bones, decreased bone mineral density (BMD), and an increased risk of fractures [1]. In recent years, it has been increasingly recognized as a serious health concern for men; estimates suggest that 6.6% of men in Europe and 6.0% of men in China suffer from the condition, respectively [2,3]. While osteoporosis, osteopenia, and fragility fractures are more common among women and older adults, the occurrence of osteopenia is lower than expected. Men are more at risk from social deprivation, but women are less affected. Between 2009 and 2012, the incidence of osteoporosis diagnoses and fragility fractures in women increased, likely due to enhanced fracture risk assessment [4]. Osteoporosis significantly impacts quality of life, particularly in physical function, with severe cases affecting quality of life. Fragility fractures are the most significant factor [5]. Osteoporosis is diagnosed using a painless, 5-to-10-minute procedure by Dual Energy X-ray Absorptiometry (DEXA) scan, measuring bone mineral density (BMD) based on bone thickness [6]. Osteoporosis is predicted by factors such as menarche age, hysterectomy history, Rheumatoid arthritis, calcium supplements, menopause age, secondhand smoking, and weight. These factors can be reduced or eliminated by lifestyle changes. Health policy makers should focus on educational programs, nutrition, and supplementation from childhood and adolescence, and reduce smoking at home [7].

When BMD drops below normal reference values but not enough to meet the diagnostic criteria for osteoporotic classification, the disease is referred to as osteopenia[1]. Although osteoporosis and osteopenia both cause decreased bone density, the severity of each condition differs. Osteopenia is characterized by a T-score between -1 and -2.5, which indicates lower-than-normal bone density, whereas osteoporosis has a T-score below -2.5, indicating a more significant loss of bone density and an increased risk of fractures [2].

Pentosidine (PTD), one of the advanced glycation end products (AGEs) in collagen, is a senescent non-enzymatic cross-link resulting from glycation and oxidation [3]. Accumulation of PTD impairs the mechanical properties of bone and is associated with brittleness of collagen Fibers [4-6]. Patients with osteoporosis, particularly those at high risk, have significant risk factors for fractures. For vertebral fractures, PTD level, weight, prevalent vertebral fracture,

and back pain were significant risk factors. PTD level was strongly associated with nonvertebral fractures due to its role in motor function decline and increased risk of falls and trauma. Pentosidine level was also associated with fracture risk in patients with severe osteoporosis following teriparatide or alendronate [13]. High PTD levels and a history of fracture in adulthood are independent risk factors for fracture occurrence in community-dwelling older adults [14]. This study aims to highlight the critical role of PTDs in osteoporosis, focusing on their impact on metabolic changes to improve diagnostic accuracy and therapeutic strategies.

2. Materials and Methods

2.1. Study Subjects

One hundred and twenty-two serum samples from Iraqi women were collected. Forty-two healthy subjects with an age mean of 52±12 were compared to 37 samples of patients with osteopenia (57±10) and 42 patients with osteoporosis (58±8) over the course of two months, from December 2023 to February 2024, in the governorate of Baghdad, Al Kadhimiya Educational Hospital and Baghdad Teaching Hospital. Based on the DEXA scan, the hospital's consulting medical staff diagnosed the patients.

2.2. Collection of Blood Samples

Approximately five milliliters of blood samples were collected from each sample and centrifuged at 3000 × g for ten minutes to separate the serum. The serum was then transferred to an Eppendorf tube and stored at -25°C.

2.3. Inclusion Criteria for samples

This study involved 121 Iraqi patients aged between 30 and 70 years, recently diagnosed with osteoporosis or osteopenia and who appeared to be in good health. These patients were attending Baghdad Teaching Hospital in the Medical City Complex and Al-Kadhimiya Educational Hospital, Iraq, during the period from December 2023 to February 2024. Bone mineral density (BMD) at the lumbar spine (L1–L4) was assessed using DEXA scans, and T-scores were recorded accordingly.

2.4. Exclusion criteria of samples

Participants with diabetes mellitus, hypertension, pregnancy, rheumatoid arthritis, cancer, thyroid disorders (both hypo- and hyperthyroidism), individuals who smoke, and those receiving steroid therapy were excluded from this study.

Dual Energy X-ray Absorptiometry

Osteopenia and osteoporosis were diagnosed according to the World Health Organization's (WHO) criteria for diagnosing osteoporosis[7]. The diagnostic test is highly accurate, requires only five to fifteen minutes to complete, and exposes patients to very little radiation. A diagnosis is made when a patient’s bone mineral density (BMD) falls below the average peak BMD of young, healthy adults of the same sex and race. This score, known as the "T-score" is the number of standard deviations (SD) below the peak young adult bone mass, which represents the bone mineral density. The T-score for osteoporosis is less than -2.5 standard deviations from the mean value of peak bone mass.

• At least one fracture and a T-score ≤ -2.5 standard deviations below the mean value of peak bone mass indicate severe osteoporosis.

A bone mineral density T-score of -1 to -2.5 standard deviations below the mean value of peak bone mass is indicative of osteopenia.

• A BMD more than -1 standard deviation below the mean value of peak bone mass is considered normal bone density.

2.3. Anthropometric Measurements

Weight (Kg) and height (cm) were recorded for all participants. The body mass index (BMI) was computed by dividing the square of height (m2) by the weight (kg) [8].

2.4. Laboratory Tests

The PTD and vitamin D3 were evaluated utilizing an enzyme-linked immunoassay kit (My Bio Source, U.S.A. and AFIAS Vitamin D Neo), respectively. Serum total cholesterol was estimated by enzymatic colorimetric tests with cholesterol oxidase and cholesterol esterase, while serum triglyceride was evaluated by the enzymatic colorimetric tests with glycerol phosphate oxidase. High-density lipoprotein cholesterol (HDL) was evaluated after precipitation of the Apo lipoprotein B-containing lipoproteins with phosphotungstic acid. Low density lipoprotein cholesterol (LDL), Very low density lipoprotein cholesterol (VLDL), Calcium, Magnesium and phosphorus were determined using an enzymatic colorimetric method (Agappe. swiss). The LDL and VLDL were also calculated using the equation:
VLDL = Triglyceride \5
LDL= (Total Cholesterol) - (VLDL+HDL) [9]

2.5. Analytical Statistics

The statistical analysis was carried out using SPSS Statistical Package (Version 27: SPSS). The one-way ANOVA was used to compare the quantitative variables between the research groups, and Pearson's analysis to find out the correlations between parameters. Receiver Operator Curve (ROC) was used to analyze the data of this study. Statistics with a p-value of less than 0.05 and 0.001 were considered significant and highly significant, respectively.

3. Results

One hundred and twenty-one participants in our study were split up into three groups: osteopenia group (OS), (n=37), osteoporosis group (OP), (n=42) and the control group (n=42). The general anthropometric of the participants are presented in Table 1. Although there was no significant difference in height, weight, or BMI between the control, OP and OS groups, the statistical ANOVA revealed a highly significant difference in BMD between the control and OP groups as well as between the OP and OS groups ($P < 0.001$).

Table1: Statistical analysis of the studied groups (osteoporosis, osteopenia, control) distribution according to height, weight, BMI and BMD.

Parameters	Control group(No.=42) (Mean ±SD)	Osteopenia group(No.=37) (Mean ±SD)	Osteoporosis group(No.=42) (Mean ±SD)	P-value		
				***P*a**	***P*b**	***P*c**
Height	160.09 ± 5.32	159.54 ± 5.53	157.6429 ± 6.15	0.902	0.123	0.305
Weight	78.3 ± 14.96	79.67 ± 11.51	74.5 ± 14.12	0.898	0.412	0.219
BMI	30.53 ± 5.66	31.4 ± 4.95	29.9 ± 5.32	0.746	0.874	0.450
BMD(g/cm^2)	1.14 ± 0.13	0 .83 ± 0.08	0.69 ± 0.08	<0.001	<0.001	<0.001

***P*a** = the osteopenia group compared to the control group, ***P*b** =the osteoporosis group compared to the control group, ***P*c** = the osteoporosis group compared to the osteopenia group.

Table 2 presents a comparative analysis of various biochemical parameters (PTD, V. D3, Ca., Mg., Ph., and lipid profile) among three groups: Control (n=42), Osteopenia (n=37), and Osteoporosis (n=42). Although PTD was significantly elevated in both the osteopenia and osteoporosis groups compared to the control ($p < 0.001$), there is no significant difference between osteopenia and osteoporosis ($p = 0.961$). These results indicate that PTD accumulation

is associated with bone loss but does not differentiate between osteopenia and osteoporosis groups. Regarding vitamin D3, drastically reduced in osteopenia (9.33 ± 3.28) and osteoporosis (8.98 ± 3.82) compared to controls (33.24 ± 1.04, $p < 0.001$) and no significant difference between osteopenia and osteoporosis ($p = 0.860$). That suggests vitamin D3 deficiency is common in both conditions but does not differentiate severity.

As for Ca, it is severely reduced in osteopenia (5.45 ± 1.77) and osteoporosis (6.4 ± 1.68) compared to controls (9.32 ± 0.50, $p < 0.001$), while osteoporosis shows a significantly higher calcium level than osteopenia ($p = 0.010$), possibly due to compensatory mechanisms or disease progression. Although there was no significant difference in Mg between control and osteopenia groups ($p = 0.716$), a significantly lower value was observed in osteoporosis compared to osteopenia ($p = 0.015$), indicating its potential involvement in disease progression. In addition, phosphorus appeared with no significant differences among the three groups. Concerning the lipid profile, TG, LDL, VLDL, and cholesterol were significantly higher in osteopenia and osteoporosis compared to the control group ($p < 0.001$), but there was no significant difference between osteopenia and osteoporosis. That suggests a link between bone density loss and lipid metabolism disorders. High density lipoprotein level was significantly lower in osteopenia compared to the control group ($p = 0.042$) but significantly higher in osteoporosis compared to osteopenia ($p < 0.001$), which suggests a complex lipid profile association with bone density changes.

Table 2: Statistical analysis of the studied groups (osteoporosis, osteopenia, control) distribution according to biomarker and diagnostic parameters (PTD, vitamin D3, Ca, Mg, Ph and lipid profile).

Diagnostic Parameters	Control group(No.=42) (Mean ±SD)	Osteopenia group(No.=37) (Mean ±SD)	Osteoporosis group(No.=42) (Mean ±SD)	P-value		
				***P*a**	***P*b**	***P*c**
PTD(ng/ml)	41.63 ± 12.53	504.35 ± 87.44	499.84 ± 94.96	<0.001	<0.001	0.961
V.D3(ng/ml)	33.24 ± 1.04	9.33 ± 3.28	8.98 ± 3.82	<0.001	<0.001	0.860
Ca(mg/dl)	9.32 ± 0.50	5.45 ± 1.77	6.4 ± 1.68	<0.001	<0.001	0.010
Mg(mg/dl)	1.96 ± 0.08	2.00 ± 0.37	1.84 ± 0.19	0.716	0.90	0.015
Ph(mg/dl)	3.56 ± 0.1	3.48 ± 0.76	3.76 ± 0.68	0.824	0.245	0.084
TG(mg/dl)	185.63 ± 15.78	206.13 ± 34.97	216.59 ± 13.99	<0.001	<0.001	0.112
LDL(mg/dl)	95.94 ± 6.13	114.49 ± 10.5	109.13 ± 12.68	<0.001	<0.001	0. 053
VLDL(mg/dl)	37.12 ± 3.15	41.22 ± 6.99	43.31 ± 2.79	<0.001	<0.001	0.112
HDL(mg/dl)	35.44 ± 2.79	30.84 ± 8.93	38.93 ± 11.04	0.042	0.137	<0.001
Cholesterol(mg/dl)	168.51 ± 6.84	186.56 ± 8.15	191.38 ± 11.58	<0.001	<0.001	0.054

***P*a** = the osteopenia group compared with the control group, ***P*b** =the osteoporosis group compared with the control group, ***P*c** = the osteoporosis group compared with the osteopenia group. PTD=Pentosidine, V. D3=vitamin D3, Ca=calcium, Mg=magnesium, Ph=phosphorus, TG=Triglycerides, LDL= low-density lipoprotein, VLDL=very low-density lipoprotein, HDL= high-density lipoprotein.

The correlation between PTD and other parameters in the osteopenia and osteoporosis groups.

Table 3 presents the Pearson correlation analysis for the osteopenia group, revealing significant negative correlations between PTD and both triglycerides (TG) and very-low-density lipoprotein (VLDL) ($r = -0.339$, $p = 0.040$). This suggests that elevated TG and VLDL levels

are linked with lower bone density in osteopenia. Other parameters (V. D3, Ca, Ph, LDL, HDL, Mg, and cholesterol) showed no significant correlation with PTD in the osteopenia group. As for the Pearson correlation of PTD with other parameters in the osteoporosis group, a statistically significant positive correlation was observed between the LDL level with PTD (r = 0.312, p = 0.045). However, there was a highly significant negative correlation between PTD with HDL (r = -0.401, p = 0.009). Other parameters (V. D3, Ca, Mg, Ph, TG, VLDL, and cholesterol) had no statistically significant correlations with PTD, as shown in Table 3

Table3: Correlation of the PTD and other parameters for the osteopenia and osteoporosis groups.

	osteopenia		**osteoporosis**	
Parameters	**r**	**P-value**	**r**	**P-value**
PTD(ng/ml)	1	-	1	-
V.D3(ng/ml)	0.005	0.976	0. 188	0.232
Ca(mg/dl)	-0.235	0.161	0.079	0.619
Mg(mg/dl)	0.277	0.097	-0.035	0.827
Ph(mg/dl)	-0.116	0.494	-0.136	0.389
TG(mg/dl)	-0.339*	0.040	0.133	0.400
LDL(mg/dl)	-0.080	0.638	0.312*	0.045
VLDL(mg/dl)	-0.339*	0.040	0.133	0.400
HDL(mg/dl)	0.295	0.076	-0.401**	0.009
Cholesterol(mg/dl)	-0.070	0.681	-0.009	0.955

r =Pearson coefficient. p>0.05= statistically non-significant correlation, * Statistically significant correlation at p< 0.05, ** Statistically significant correlation at p≤0. 01.V. D3=vitamin D3, Ca=calcium, Mg=magnesium, Ph=phosphorus, TG=Triglycerides, LDL= low-density lipoprotein, VLDL=very low-density lipoprotein, HDL= high-density lipoprotein.

Receiver operating characteristic (ROC) curve of PTD

The ROC curve of PTD showed an area under the curve (AUC) of 1.000 for both osteopenia and osteoporosis, showing that PTD has cutoff values of 184.2000 for osteopenia and 177.8500 for osteoporosis, indicating a powerful potential marker for these conditions with cutoff values as detailed in Table 4 and illustrated in Figure 1.

Figure1 (A): Receiver operator curve analysis for the predictive value of PTD levels for the people which suffering from osteopenia group (n=37) versus healthy groups (n=42), AUC= area under the curve, SE= standard error, CI= confidence interval,(B): The Receiver operator curve analysis for the predictive value of PTD levels for the people which suffering from osteoporosis group (n=42) versus healthy groups, AUC= area under the curve, SE= standard error, CI= confidence interval.

Table 4: The Receiver operator curve data for PTD as biomarker parameter for the osteopenia and osteoporosis diseases.

Biomarker Parameter	AUC	SE	95% CI	Specificity (%)	Sensitivity (%)	Cut-off value	P-value
PTD for OP	1.000	0.0001	1.000	0.000	1.000	184.2000	<0.001*
PTD for OS	1.000	0.0001	1.000	0.000	1.000	177.8500	<0.001*

AUC= area under the curve, SE= standard error, CI= confidence interval, OP=osteopenia, OS=osteoporosis, PTD=Pentosidine. * highly Significant at p < 0.001.

The ROC curve for PTD showed an AUC of 1.000 for both osteopenia and osteoporosis groups, indicating perfect sensitivity and specificity. The cutoff values were identified as 184.2000 pg/mL for osteopenia and 177.8500 pg/mL for osteoporosis.

4. Discussion

The silent disease (OP) is characterized by low bone mass, which is caused by bone loss and insufficient bone formation. This causes it to become more brittle, increasing the chance of fractures, particularly in the areas of the spine, hip, wrist, and shoulder [10]. When intracellular OS is linked to inflammatory cytokines, it promotes osteoclast bone resorption while suppressing osteoblast bone formation [11, 12]. Since this study was the first to be done on Iraqi patients and it connected osteoporosis to the biomarker PTD, it is unique in the country.

This study examined the connections among different biochemical markers, osteoporosis, osteopenia, and bone health. The results augment existing knowledge about the complex etiology of skeletal diseases and their candidate diagnostic markers. There were no significant differences in height, weight, or BMI among the control, osteopenia, and osteoporosis groups, indicating that these parameters do not strongly correlate with bone health status. As shown, the study design has considerable strength with three well-defined groups, Control (n=42), Osteopenia (n=37) and Osteoporosis (n=42). The age stratification reveals an important epidemiological pattern: Control (51.5 ± 11.95 years), Osteopenia (57.21 ± 10.75 years) and Osteoporosis (58.45 ± 8.63 years). Groups with osteopenia and osteoporosis are consistent with the known fact that age is a significant risk factor for bone loss [13].
While the results of Anthropometric Parameters and their Clinical Significance showed the lack of significant BMI differences ($p>0.05$), with no statistically significant differences control (30.53 ± 5.66), Osteopenia (31.4 ± 4.95) and Osteoporosis (29.9 ± 5.32) across groups challenges the traditional assumption about weight's protective effect against bone loss. This implies that although diseases such as osteopenia and osteoporosis cause alterations in bone density and composition, they may not have an impact on total body mass or the fat-to-height ratio, which is measured by BMI [14, 15]. These results suggest that, although pertinent, traditional anthropometric measures might not be as accurate indicators of bone health as biochemical and molecular markers. While BMD values show a clear stepwise decline in Control (1.14 ± 0.13), Osteopenia (0.83 ± 0.08) and Osteoporosis (0.69 ± 0.08). However, it showed a highly significant decline in both the osteopenia and osteoporosis groups compared to the control group ($P < 0.001$).

This decline was more pronounced in the osteoporosis group. BMD remains a cornerstone diagnostic marker for differentiating healthy individuals from those with osteopenia or osteoporosis. Its significant decrease highlights the progressive nature of bone density loss in these conditions. Additionally, earlier research has demonstrated [16],[17].

Diagnostic Biomarkers or Metabolic Parameters, Vitamin D3 levels were significantly lower in both osteopenia and osteoporosis groups compared to controls ($P < 0.001$). This supports the well-established role of vitamin D deficiency in impaired bone metabolism, Control group (33.24 ± 1.04), Osteopenia group (9.33 ± 3.28) and Osteoporosis (8.98 ± 3.82). Because it helps the intestines absorb calcium and phosphorus and controls bone remodelling by osteoblasts (cells that make new bone) and osteoclasts (cells that break down existing bone), vitamin D3 (cholecalciferol) is essential for maintaining bone health [18]. Fascinating, Magnesium levels showed significant variation between osteopenia and osteoporosis ($P = 0.015$), highlighting its potential role in bone health differentiation. This is consistent with previous studies [19, 20].

And a lower serum magnesium value was discovered in another study; also, a significant risk of fractures was directly linked to the Mg concentration [21, 22].
In order to reduce the incidence of bone problems, these findings emphasize the need to maintain adequate amounts of essential minerals. Additionally, our research indicates that magnesium may be a secondary factor but has a significant role in the development of osteoporosis, meriting further study.
Phosphorus, in conjunction with calcium, is necessary for the formation of hydroxyapatite, the mineralized component of bones [22]. It promotes metabolic processes, such as energy transfer (via ATP) and cell signaling, as well as structural integrity [23]. Phosphorus levels showed no significant differences across the groups. However, when it comes to bone-related disorders, phosphorus is usually less stressed than calcium. And in both osteopenia and osteoporosis groups, there were no correlations between PTD and phosphorus in osteopenia and osteoporosis groups ($r=-0.116$, -0.136, respectively; $p > 0.05$). The data reaffirm the critical interplay between vitamin D3, calcium, and magnesium in maintaining bone health. The progressive decline in these parameters corresponds to worsening bone density.

A lipid profile is a diagnostic tool that assesses the blood's concentrations of different lipids, such as triglycerides and cholesterol. Osteopenia and osteoporosis groups exhibited significantly higher levels of cholesterol, triglycerides (TG), LDL, and VLDL compared to controls. High-density lipoprotein was significantly lower in these groups. Interestingly, HDL levels were markedly higher in osteoporosis compared to osteopenia ($P < 0.001$).

Dyslipidemia appears to be a contributing factor or associated marker of poor bone health. The observed patterns suggest lipid metabolism alterations in osteopenia and osteoporosis, which could serve as potential therapeutic targets. Additionally, these results are consistent with the data published by hadiri-Anari A et al [23] and other studies [24, 25]. This leads us to believe that lipids, especially triglycerides and cholesterol, affect bone health by way of inflammatory pathways. Dyslipidemia-induced chronic inflammation can interfere with bone remodelling processes, resulting in a reduction in BMD. This study highlights the fact that people with osteopenia and osteoporosis have lower BMD and higher lipid levels, indicating the same pathophysiological process.

As for PTD, this study found PTD to be a highly discriminant biomarker for osteoporosis and osteopenia. Both osteopenia and osteoporosis groups exhibited significantly elevated PTD levels compared to controls ($P < 0.001$). The study showed the relationship between PTD and lipid profile, a significant negative correlation between PTD and TG in the osteopenia group, suggesting a protective role of elevated TG against osteopenia. A significant positive correlation between PTD and LDL in the osteoporosis group indicates that higher LDL levels may exacerbate osteoporosis risk. A highly significant negative correlation between PTD and HDL in the osteoporosis group ($P < 0.001$), highlighting a protective role of HDL.

As for the Correlation between PTD and other Parameters, the correlation analysis: no significant relationship between PTD and vitamin D3, calcium, magnesium, or cholesterol levels in both osteopenia and osteoporosis groups. But significant associations between PTD and lipid profile parameters (TG, LDL, HDL) in specific groups, pointing to lipid metabolism as an important factor in bone health.

As for the Receiver Operating Characteristic (ROC) curve for PTD showed an Area under the Curve (AUC) of 1.000 for both osteopenia and osteoporosis, with ($p<0.001$). Confirming PTD's high sensitivity and specificity as a diagnostic biomarker. The cutoff values identified were: 184.2000 pg/mL for osteopenia and 177.8500 pg/mL for osteoporosis. PTD emerges as

a robust diagnostic biomarker for distinguishing between healthy bone status, osteopenia, and osteoporosis. Its high diagnostic potential underscores its value in early detection and monitoring. This aligns with a prior study [24, 26, 27].
Future studies should focus on longitudinal studies to examine causal relationships and validate the diagnostic utility of PTD. New therapeutic targets may be found by investigating the mechanisms underlying the relationship between lipids and bone, as well as the role of magnesium in bone metabolism.

Conclusion

This study identifies Pentosidine (PTD) as a highly sensitive and specific biomarker for differentiating between osteopenia and osteoporosis, with an AUC of 1.000 for both conditions. The results demonstrate strong associations between bone health deterioration and reduced vitamin D3 levels, decreased BMD, dyslipidemia, and magnesium imbalance. These results advocate for the inclusion of PTD in diagnostic protocols and emphasize the need for further longitudinal studies to validate its clinical utility and explore lipid and mineral metabolism as therapeutic targets.

6. Ethical Clearance

The Research Ethics Committee for Scientific Research has been approved by the College of Science Ethics Committee at the University of Baghdad (Ref.CSEC/1123/0125 on November 26,2023).

7. Acknowledgment

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8. Disclosure and conflict of interest

There is no conflict of interest.

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