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Original article

Serum osteocalcin concentration as a biomarker of osteoporosis in Egyptian patients with chronic renal failure on regular hemodialysis

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Abstract

Background: To prevent and treat mineral bone damage in those with long-term kidney failure [CKD-MBD], early detection and intervention are crucial. Osteocytes, odontoblasts, and osteoblasts create a protein called osteocalcin [OC], which is dependent on vitamins K and D. It is present in serum and deposited in the extracellular bone matrix. Serum osteoblast activity and bone formation rate are thought to be indicated by serum OC hydrolyzed in the kidney and liver. It might impact mineralization, bone turnover, and osteoblast function control. **Aim and Objectives:** to assess the serum osteocalcin levels in Egyptian patients receiving regular hemodialysis as a biomarker of osteoporosis due to chronic renal failure. **Patients and methods:** The Beni-Suef University Hospital's internal medicine department and hemodialysis unit were the sites of this case-control research.

In addition to 45 healthy controls, 45 CRF patients were included in this research. **Results:** When comparing Dexa results, kidney function tests, osteocalcin levels, correlations between osteocalcin levels, and other laboratory investigations, the study found a statistically significant difference in the sensitivity and specificity of osteocalcin levels in the diagnosis of osteoporosis CRF cases from controls. Specifically, there was a significantly lower GFR [mean 7.09] and a significantly higher level of osteocalcin [mean 62.1] with a p-value <0.05 among cases compared to controls [mean 29.6]. These findings concluded that osteocalcin levels were elevated in CRF patients with osteoporosis. **Conclusion:** Osteocalcin may be a useful biomarker for osteoporosis early detection ing patients receiving frequent hemodialysis and chronic renal failure. According to the study, serum osteocalcin measurement may be utilized as a potential supplementary biomarker in conjunction with a Dexa scan for the early identification of CKD-MBD and the tracking of osteoporosis progression in clinical settings.

1. Introduction :

To prevent and treat mineral bone damage in those with long-term kidney failure [CKD-MBD], early detection and intervention are crucial. Osteocytes, odontoblasts, and osteoblasts create a protein called osteocalcin [OC], which is dependent on vitamins K and D. It is present in serum and deposited in the extracellular bone matrix. Serum osteoblast activity and bone formation rate are thought to be indicated by serum OC hydrolyzed in the kidney and liver. It might impact mineralization, bone turnover, and osteoblast function control [1]. Characteristics of the skeletal disease osteoporosis include weakened bones and an increased risk of fractures. Bone density and quality are interdependent, and this is seen in bone

strength. The factors that influence bone density include peak bone mass and the extent of bone loss. [2].

Risk factors for fragility fractures, osteoporosis, and mineral bone disorder [MBD] include chronic kidney disease [CKD]. [3].

Chronic renal disease with mineral and bone metabolism complications is characterized by a systemic illness that may be identified by the following symptoms: calcium, phosphorus, parathyroid hormone [PTH], and vitamin D metabolism problems; anomalies in bone turnover, mineralization, volume, linear growth, and strength; and calcification of arteries and other soft tissues. Elevated risk of fracture, increased morbidity, and higher mortality are among the effects of CKD-MBD [4].

These days. Osteoporosis is primarily diagnosed using bone densitometry [DEXA] to assess bone mass [5].

The process of remodeling bone produces biomarkers related to bone creation, resorption, and turnover regulation. Biomarkers of proteins, enzymes, and byproducts throughout the process of bone remodeling have been used to study the detection of bone metabolism [6].

When DXA's BMD value is insufficient to diagnose osteoporosis, these indicators can help provide an early assessment of the condition. Thus, combining bone biomarker detection and DXA-measured BMD indicates considerable promise for improving the early assessment of those at high risk for osteoporosis. Finding alternative biomarkers that could offer a sensitive and quick way to track the development of CKD-MBD has drawn increasing attention [7]. Osteocalcin is a collagen-free, glutamate-rich polypeptide bone matrix protein having 49 amino acids. Osteocalcin is made by osteoblasts and incorporated into the matrix of the bone. Osteocalcin is regarded as a sign of bone turnover rather than a particular indication of bone production since it is released into the circulation from the matrix during bone resorption [8].

Measurement of plasma Recent research has suggested that osteocalcin may be a biomarker for the early detection of CKD-MBD. This study aimed to evaluate serum osteocalcin levels in CRF patients as a standard early marker of osteoporosis.

2. Patients And Methods :

The Beni-Suef University Hospital's hemodialysis unit and internal medicine department were the study's sites. In addition to 45 healthy controls, 45 CRF patients were included in the study.

Inclusion criteria

Individuals were receiving regular hemodialysis and those with stage 5 chronic kidney disease [e GFR: < 15].

Exclusion criteria

Patients with acute kidney injury, age less than 18 years, rheumatic heart disease, and other bone diseases.

Ethical considerations: Provide patients complete information about the study and the marker. Every participant included in the study gave their written, informed permission. Approval of the research ethics committee of Beni-Suef University was taken. Approval No: FMBSUREC/09042023/Eid

Methodology

1. The patients underwent a thorough history taking that included information on their age, smoking status, length of hemodialysis, cause of end-stage renal disease [ESRD], medication history, current course of therapy, and any related comorbidities such as diabetes mellitus, hypertension, chronic vascular disease, and surgical history.
2. Complete clinical examination and measurement of the systolic and diastolic blood pressure.
3. Routine laboratory investigations include CBC, Serum calcium, Serum phosphorus, Kidney function tests [creatinine, urea], PTH, and Alkaline phosphatase.
4. Radiological tests :
   - Dual-energy X-ray absorptiometry [DEXA SCAN].
   - X-ray abdomen lateral view.
5. Special tests:
   - Serum osteocalcin level by enzyme-linked immunoassay [ELIZA].

Test principle

Using a double-antibody sandwich enzyme-linked immunosorbent assay [ELISA], this kit determines the Human Osteocalcin-Bone Gla protein [OT/BGP] concentration in samples.

Add biotin-labeled Osteocalcin-Bone Gla protein antibodies and mix them with Streptavidin-HRP to create an immunological complex after pre-coating the enzyme well with Human Osteocalcin-Bone Gla Protein. Monoclonal antibody and letting it incubate. Lastly, keep the incubation going. To remove the enzyme that had separated, and then wash again. The liquid becomes blue after adding Chromogen Solutions A and B. The color changes to yellow as a result of the acidic action.

Statistical Analysis:

- All data was input twice into Microsoft Access after collection and coding to make data manipulation easier. SPSS, a statistical

package developed by SPSS Inc. of Chicago, IL, USA, was used for data analysis.

- Standard deviations and means for quantitative parametric data and simple descriptive statistics for qualitative data are presented as percentages and numbers.
- The study's quantitative data was subjected to a one-sample Kolmogorov-Smirnov test to ensure normality in each group before being subjected to inferential statistic testing.

➢ **For quantitative parametric data**

- Random samples: Two separate groups were compared quantitatively using a t-test.

➢ **For quantitative non parametric data**

- **Two separate groups were compared using the Mann-Whitney U test.**

➢ **For qualitative data**

- When comparing more than two qualitative groups, the chi-square test is used.
- We used the bivariate Spearman's correlation test to examine the relationship between quantitative non-parametric variables.
- The "Receiver Operating Characteristic" [ROC] curve is used to assess the sensitivity and specificity of the marker.

❖ Statistical significance was determined by a P-value< 0.05.

3. Results:

According to the table, there was a statistically significant **lower** level of GFR and a significantly **higher** level of creatinine, urea, phosphorus, PTH, ALP, and Osteocalcin with p-value <0.05 among cases when compared to controls. On the other hand, there was no statistical significance difference among cases and controls regarding calcium levels with a p-value >0.05.

Table [1]: Comparisons of laboratory investigations in different study groups.

Variables	Cases [N=45]		Control [N=45]		P-value	Sig.
	Mean	SD	Mean	SD		
Kidney function tests						
Creatinine	**8.8**	2.9	0.74	0.16	**<0.001**	**HS**
Urea	**114.5**	42.4	32.4	6.5	**<0.001**	**HS**
GFR	7.09	4.1	108.1	18.9	**<0.001**	**HS**
Bone labs						
Calcium	9.2	1.2	8.9	0.51	0.16	NS
Phosphorus	**4.5**	1.2	4.1	0.61	**0.03**	**S**
PTH	**345.5**	346.03	28.9	7.2	**<0.001**	**HS**
ALP	**235.1**	203.7	73.2	13.2	**<0.001**	**HS**
Osteocalcin	**62.1**	57.2	29.6	35.2	**0.002**	**HS**

The table illustrated a statistically significant lower mean of DEXA findings [spine, femur, and forearm] between cases in contrast to controls with p-value <0.001.

Table [2]: Comparisons of DEXA findings in different study groups.

Variables	Cases [N=45]		Control [N=45]		P-value	Sig.
	Mean	SD	Mean	SD		
DEXA -Spine	0.34	2.6	3.8	0.83	**<0.001**	**HS**
DEXA -Femur	-0.68	1.5	3.7	0.87	**<0.001**	**HS**
DEXA -Forearm	-2.7	1.8	3.9	0.87	**<0.001**	**HS**

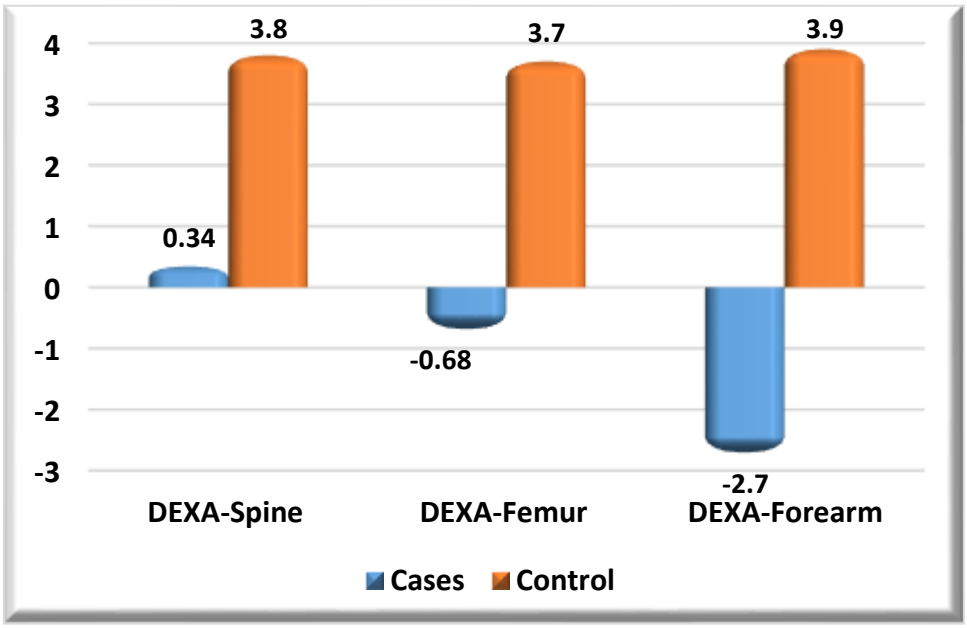

Figure [1] Comparisons of DEXA findings in different study groups.

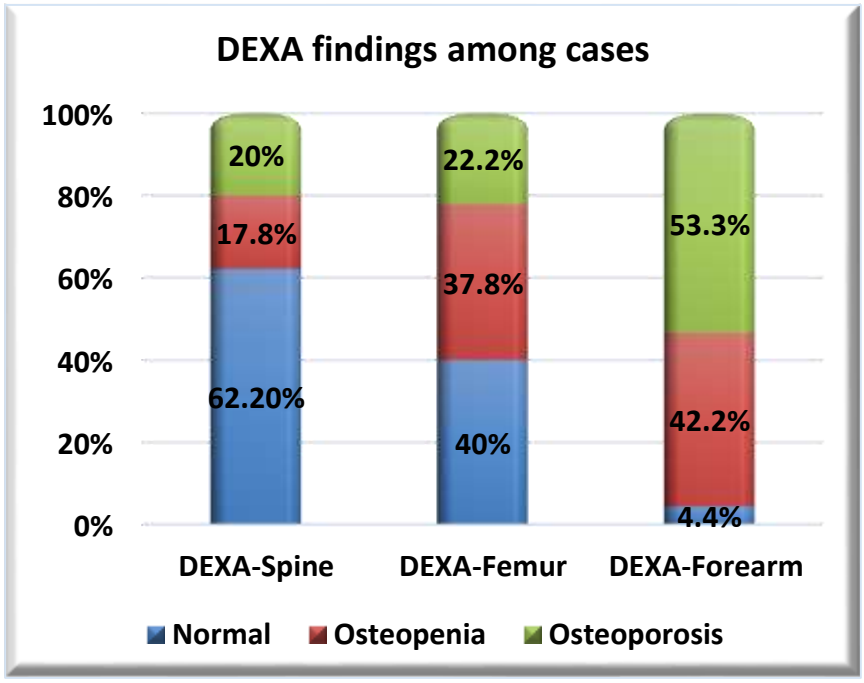

Figure [2] Comparisons of radiological findings in different study groups**.**

The table illustrated a statistically significant AAC in the X-ray abdomen and a higher percentage of osteopenia and osteoporosis among cases in DEXA [spine, femur, and forearm] with p-value <0.001].

Table [3]: Comparisons of radiological findings in different study groups.

Variables	Cases [N=45]		Control [N=45]		P-value	Sig.
	No.	%	No.	%		
X-ray abdomen						
Normal	22	48.9%	45	100%	**<0.001**	**HS**
AAC	23	51.1%	0	0%		
DEXA Spine						
Normal	28	62.2%	45	100%	**<0.001**	**HS**
Osteopenia	8	17.8%	0	0%		
Osteoporosis	9	20%	0	0%		
DEXA femur						
Normal	18	40%	45	100%	**<0.001**	**HS**
Osteopenia	17	37.8%	0	0%		
Osteoporosis	10	22.2%	0	0%		
DEXA forearm						
Normal	2	4.4%	45	100%	**<0.001**	**HS**
Osteopenia	19	42.2%	0	0%		
Osteoporosis	24	53.3%	0	0%		

The table illustrated that the DEXA spine, femur, and forearm show a statistically significant **positive** correlation with GFR level and a statistically significant **negative** correlation with creatinine and urea levels with a p-value <0.05.

Table [4]: Correlation between DEXA findings and routine laboratories among the study groups.

Variables	DEXA findings					
	Spine		Femur		Forearm	
	R	P-value	R	P-value	R	P-value
Kidney function tests						
Creatinine	-0.66	**<0.001***	-0.77	**<0.001***	-0.82	**<0.001***
Urea	-0.57	**<0.001***	-0.72	**<0.001***	-0.80	**<0.001***
GFR	0.67	**<0.001***	0.84	**<0.001***	0.88	**<0.001***

The table illustrated that there was a statistically significant **negative** correlation with p-value <0.05 between all DEXA findings [spine, femur, and forearm] with all bone lab investigations [calcium, phosphorus, PTH, ALP, and osteocalcin levels], which indicated a decrease in all these labs will be associated with an increase in DEXA measures.

Table [5]: Correlation between DEXA findings and bone laboratories among the study groups.

Variables	DEXA findings					
	Spine		Femur		Forearm	
	R	P-value	R	P-value	r	P-value
Calcium	0.02	0.85	-0.06	0.52	-0.07	0.52
Phosphorus	-0.21	**0.04***	-0.25	**0.01***	-0.30	**0.005***
PTH	-0.36	**<0.001***	-0.46	**<0.001***	-0.50	**<0.001***
ALP	-0.32	**0.002***	-0.47	**<0.001***	-0.56	**<0.001***
Osteocalcin	-0.25	**0.01***	-0.31	**0.003***	-0.31	**0.003***

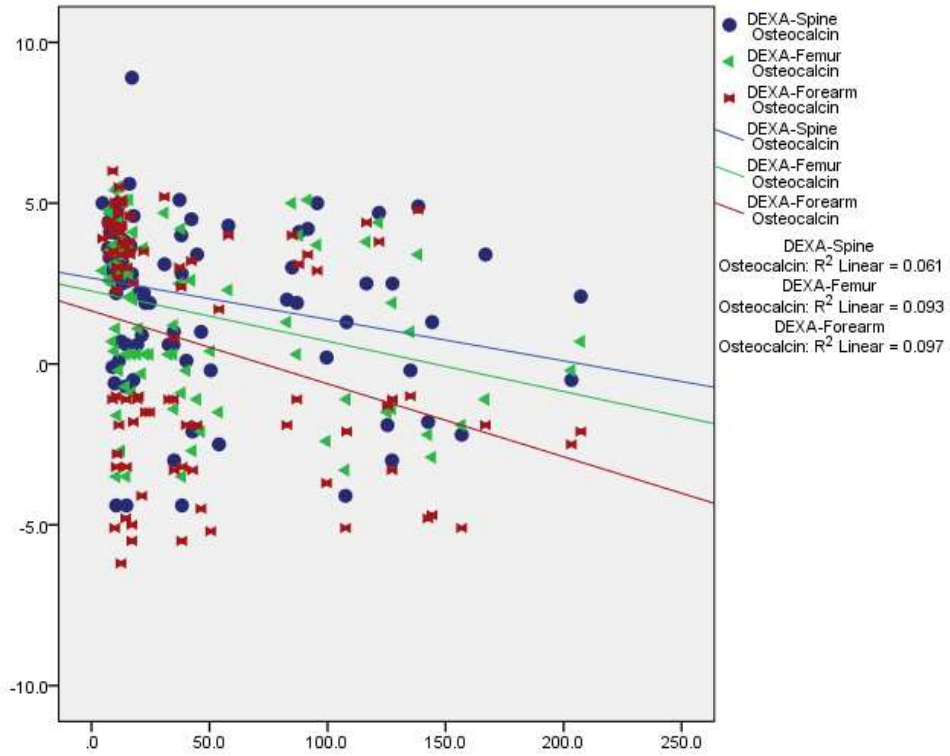

Figure [3] Correlation between DEXA findings and bone laboratories among study groups.

Table illustrated that phosphorus, PTH, ALP, and osteocalcin levels show a significant Specificity and sensitivity test in the detection of cases with a sensitivity of [66.7,95.6,88.9, and 77.8%] and a specificity of [53.3,86.7,77.8, and 68.9%] at cut off value [4.15,37.5,83.5, and 16.7] correspondingly. Yet there was no significant effect on calcium level in the diagnosis of cases with p-value >0.05.

Table [6]: Specificity and sensitivity of bone laboratory test in the detection of cases

Variable	Sensitivity	Specificity	Accuracy	Cut off point	P-Value [CI 95%]
Calcium	77.8%	11.1%	61.5%	8.3	0.06 [49-74]
Phosphorus	66.7%	53.3%	63.3%	4.15	0.02 [51.1-75.5]
PTH	95.6%	86.7%	97.5%	37.5	<0.001 [94-100]
ALP	88.9%	77.8%	86%	83.5	<0.001 [77.3-94.6]
Osteocalcin	77.8%	68.9%	74.2%	16.7	<0.001 [63.9-84.4]

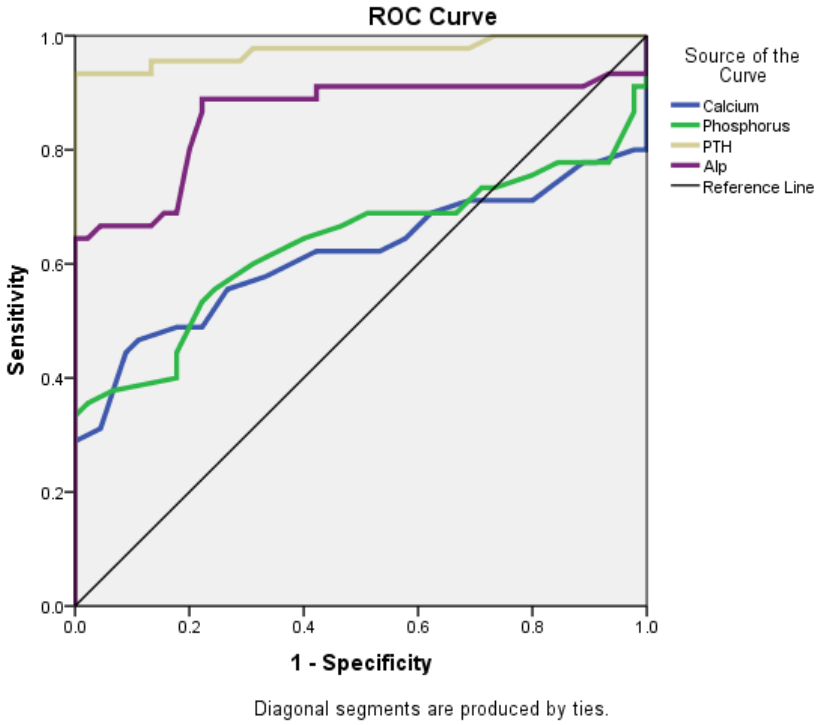

Figure [4]: ROC curve for bone lab investigations in detecting cases.

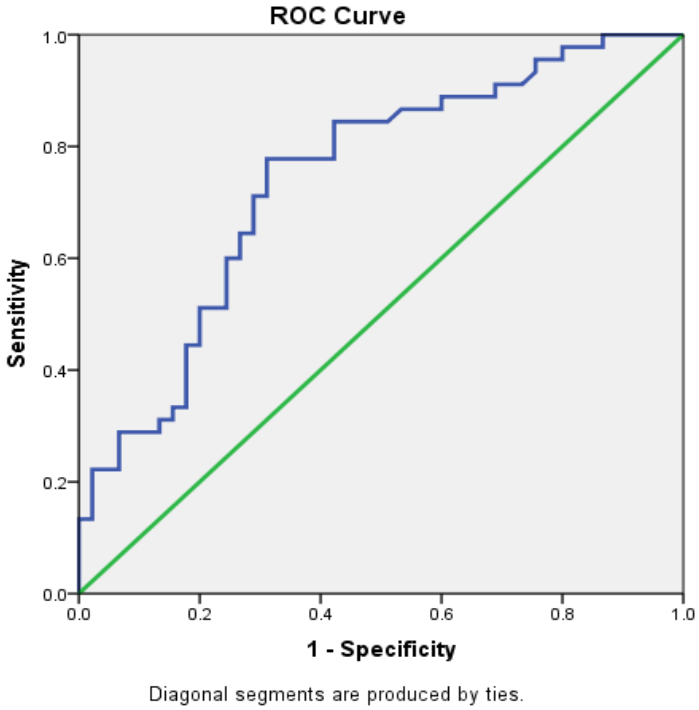

Figure [5]: ROC curve for Osteocalcin level in the detection of cases.

Table illustrated that all DEXA findings [spine, femur, and forearm] levels show a significant Sensitivity and specificity test in the detection of cases with a sensitivity of [91.1%, 100%, and 100%] and a specificity of [88.9, 100 and 100%] at cut off value [2.85, 2.05, and 2] respectively.

Table [7]: Sensitivity and specificity of DEXA findings in the detection of cases

Variable	Sensitivity	Specificity	AUC	Cut off point	P-Value [CI 95%]
Spine	91.1%	88.9%	93.7%	2.85	<0.001 [87.6-99.7]
Femur	100%	100%	100%	2.05	<0.001 [100]
Forearm	100%	100%	100%	2	<0.001 [100]

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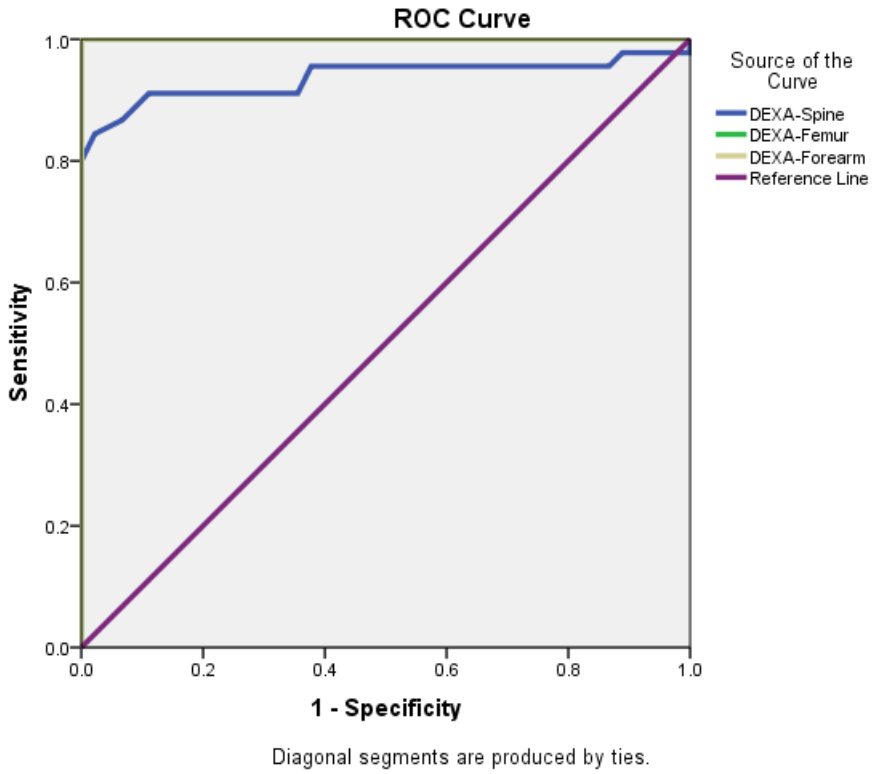

Figure [6]: ROC curve for DEXA findings in the detection of cases

4. Discussion:

Kidney Disease -Improving Global Outcomes [9] guidelines described the syndrome of CKD-mineral bone problem, which comprises soft tissue calcification, the degree of renal osteodystrophy, and conventional mineral biochemical abnormalities. These irregularities may be associated with left ventricular hypertrophy [10].

The frequency of osteoporosis varies with the stage of chronic kidney disease. In individuals with early-stage CKD [stages 1-3 A CKD], fractures are more commonly associated with typical osteoporosis than with CKD-MBD [11]. On the other hand, most patients with stage 4 or 5 CKD show signs of CKD-MBD and a degree of decreased bone mineral density [BMD] [12]. Approximately 50% of the patients may have had a fracture at the time of starting dialysis [13].

Osteocalcin, also referred to as the gamma-carboxyglutamic acid-containing protein of the bone, is an amino acid-based protein. Osteoblast-produced OC is crucial for calcium homeostasis, mineralization, and bone modulation [14].

It has been shown that there is a strong correlation between the rise in BMD and the serum OC level during osteoporosis treatment. Serum osteocalcin is considered a particular indicator of osteoblast function and a gauge of the degree of bone production in osteoporosis. Osteocalcin has been regarded in numerous research as a crucial biomarker to evaluate the effectiveness of the medication on the creation of bones. The benefits of osteocalcin as a bone production biomarker are tissue specificity, broad availability, and minimal fluctuation. Serum OC, a biomarker of bone remodeling, has potential applications in evaluating osteoporosis and fracture risk in older adults [15].

A statistically significant lower level of GFR [mean 7.09] and a considerably higher level of osteocalcin [mean 62.1] with a p-value <0.05 among cases as compared to controls [mean 29.6] indicated that the osteocalcin level was significantly elevated in CRF patients with osteoporosis. Additionally, all DEXA results [spine, femur, and forearm] showed a statistically significant negative connection with a p-value <0.05 with osteocalcin levels, suggesting that an increase in DEXA measures will be linked to a drop in osteocalcin.

When comparing the spine, femur, and forearm DEXA findings between patients and controls, there was a statistically significant decrease in mean [p-value

<0.001] among the cases. Among cases in DEXA [spine, femur, and forearm], there was a higher percentage of osteopenia and osteoporosis with a statistically significant AAC [p-value <0.001]. Additionally, there was a statistically significant negative connection [p-value <0.05] between all bone lab investigations [calcium, phosphorus, PTH, and ALP levels] and all DEXA findings [forearm, femur, and spine]. This suggested that an increase in DEXA measures would correlate with a drop in all these laboratories. We also evaluated the specificity and sensitivity of osteocalcin in the diagnosis of osteoporosis in CRF patients. Osteocalcin level shows a significant sensitivity and specificity test in the detection of cases with a sensitivity of [77.8%] and a specificity of [68.9%] at the cut-off value [16.7]. Additionally, a significant sensitivity and specificity test in the detection of cases is shown by all DEXA results [spine, femur, and forearm] at cut-off values [2.85, 2.05, and 2], with a sensitivity of [91.1%, 100%, and 100%] and specificity of [88.9, 100 and 100%]. Phosphorus, PTH, and ALP level sensitivity and specificity tests demonstrate a significant sensitivity and specificity test in case detection, with sensitivity values of 66.7, 95.6, and 88.9.

These findings were consistent with a study by Po Jui Chi et al. [2022] [16], which assessed serum osteocalcin. The osteoporosis group was found to have higher phosphorous levels, calcification scores, osteocalcin levels, and intact parathyroid hormone [PTH] levels. Serum osteocalcin was positively correlated with intact PTH in a multivariate linear regression model, suggesting that osteocalcin may be a bone turnover marker in patients with chronic kidney disease [CKD]. BMD was measured using dual-energy X-ray absorptiometry. Abdominal aortic calcifications[AAA] were generated from lateral lumbar radiograph findings. However, it contradicted our conclusions about the osteoporosis group's intact PTH. Additionally, this is consistent with a study conducted by Soheir A. Ellakany, MD, et al. 2019 [17], which measured the serum phosphorus, magnesium, calcium, alkaline phosphatase [ALP], iPTH, and OC. It was shown that hemodialysis patients with end-stage renal disease [ESRD] had a notably higher serum OC level than controls. Serum OC was considerably lower in patients with adynamic bone disease who underwent parathyroidectomy than in the equivalent subgroup. In the whole patient sample, there were statistically significant positive

relationships between serum OC, total ALP, and iPTH.

Also, the undercarboxylated osteocalcin [ucOC] was examined in the Medine Alpdemir et al. 2021[18] study as a potential predictor of bone turnover in patients receiving hemodialysis [HD] and peritoneal dialysis [PD]. Additionally, they have investigated the connections between ucOC levels and other markers of bone health, including osteocalcin [OC], vitamin D, calcitonin, intact parathyroid hormone [iPTH], calcium [Ca], phosphate [P], magnesium [Mg], and bone mineral density [BMD]. The findings demonstrated that both the HD and PD groups had elevated ucOC levels in chronic kidney disease [CKD]. Compared to the control group, the ucOC levels for CRF were greater and statistically significant. Positive correlations were found between ucOC levels and OC, B-ALP, ALP, iPTH,

Kuźniewski et al.'s 2016 study [19] aimed to determine if patients' higher levels of bone markers are linked to disturbed bone metabolism in patients with chronic kidney disease [CKD]. The study aimed to assess the associations between energy metabolism indices and serum concentrations of bone markers in patients with chronic kidney disease [CKD] receiving hemodialysis. Specifically, the association between different forms of osteocalcin and adiponectin was examined. The levels of adiponectin, tartrate-resistant acid phosphatase, bone alkaline phosphatase, carboxylated, undercarboxylated, and intact osteocalcin were measured in serum. The bone markers' analysis revealed a favorable correlation between them and adiponectin. This verified that CKD patients on maintenance hemodialysis have a favorable correlation between cOC, intact OC, and adiponectin concentrations. The amounts of cOC, ucOC, and intact OC were several times greater than those recommended by the makers. However, the bone turnover indicators bALP and TRAP were only modestly raised. Furthermore, in line with the findings of our investigation, we found that the concentrations of cOC and ucOC in hemodialyzed patients were substantially greater than those in 36 healthy participants.

Conversely, in the search done by Csiky et al. [2018], [ELISA] was used to evaluate the serum concentrations of OC, OP, and OPG. According to the results consistent with our investigation, uremic patients had serum levels of the bone-related proteins OC, OPG, and OP that were several times greater than those of the controls [20].

Additionally, serum BTMs such as pro-collagen type 1 amino-terminal propeptide, β-isomerized C-terminal telopeptides, and N-terminal midfragment osteocalcin were examined in the Dengpiao Xie et al. 2023 [21] investigation. The findings demonstrated a considerable increase in BTMs between the early and advanced phases of CKD. In CKD patients, PTH levels were positively and independently correlated with BTM levels. β-CTX and N-MID OC levels were considerably greater in SHPT patients than in non-SHPT patients in the advanced stage of CKD. There was a negative correlation found between the estimated glomerular filtration rate and β-CTX, tPINP, and N-MID OC. N-MID OC, β-CTX, and tPINP were found to be independently related to PTH through multiple analyses. In comparison to patients without secondary hyperparathyroidism [SHPT], CKD patients with SHPT have higher levels of β-CTX and N-MID OC.

An electrochemiluminescence immunoassay analyzer was used to evaluate the serum levels of bone turnover indicators, intact parathyroid hormone, 25 hydroxyvitamin D, P1NP, OC, and CTX in the Junhao Lv et al. [2015] investigation. Standardized techniques were used to analyze calcium, phosphate, and serum alkaline phosphatase. Bone mineral density was measured using Dexa. Plain radiography images of the hands and pelvis and lateral abdominal radiography were used to assess vascular calcification. According to the findings, among kidney transplant candidates, the incidence of vascular calcification was 51.1%, and that of osteoporosis was 27.6%. Similar risk factors for osteoporosis and vascular calcification included advanced age, prolonged dialysis, parathyroid hyperplasia, elevated levels of iPTH, and indicators of bone turnover. Every bone turnover measure and iPTH showed significant, positive associations.
[22].

5. Conclusion:

In conclusion, our study demonstrated a statistically significant positive correlation between serum osteocalcin level and osteoporosis in Egyptian patients with chronic renal failure on regular hemodialysis. Screening osteoporosis in CRF patients on regular hemodialysis using serum osteocalcin level could be a useful and simple method.

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