The Relationship between Serum Osteopontin level and Parameters of Chronic Kidney Disease – Mineral Bone Disease in Patients on Regular Hemodialysis

Alaa M. Nawar[*1], Yasser A. A. Elhendy[2], Malak Nabil[1], May M. Sami[3], Ahmed M Salah[2], Hala M. Allam[2]
[1]Theodor Bilharz Research Institute, Cairo, Egypt
[2]Department of Internal Medicine and Nephrology, [3]Department of Clinical Pathology,
Faculty of Medicine, Zagazig University, Alsharquia, Egypt
***Corresponding author:** Alaa M Nawar, **Mobile:** (+20) 01117597723, **E-Mail:** alaamnawar1291@gmail.com

ABSTRACT

Background: Chronic Kidney Disease (CKD) is becoming a major health concern worldwide. For many patients, CKD is associated with substantial morbidity and mortality. Osteopontin (OPN) is an extracellular matrix protein first identified in bone tissue and has pleiotropic functions due to its common expression in the main organs and apparatuses. It is a phosphorylated glycophosphoprotein composed of 314 amino acids, involved in biomineralization and remodeling.

Objective: This research aimed to assess the serum level of osteopontin in patients with end-stage renal disease (ESRD) on regular haemodialysis and to correlate osteopontin level in patients with ESRD on hemodialysis with other biomarkers CKD-MBD.

Patients & Methods: This Study was conducted on 160 participants that were divided into two groups. Control group included 80 healthy subjects of both sexes, and patients group that included 80 ESRD patients on regular hemodialysis of both sexes. All studied groups were subjected to osteopontin level by enzyme-linked immunosorbent assay (ELISA).

Results: Serum osteopontin levels were higher in ESRD patients on regular dialysis than in healthy individuals, where it might have a higher predictive value for CKD development. Also, they were positively correlated with serum phosphorus, serum alkaline phosphatase and serum parathyroid hormone, which are parameters of chronic kidney disease-mineral and bone disorder.

Conclusion: Osteopontin may be considered an early marker of chronic kidney disease.

Keywords: Osteopontin, Kidney, Hemodialysis.

INTRODUCTION

End-stage renal disease (ESRD) is the fifth stage of renal failure necessitating dialysis or kidney transplantation. Chronic kidney disease leads to dysregulation of calcium (Ca^{2+}), phosphorus (P), parathyroid hormone (PTH), and vitamin D metabolism, resulting in biochemical laboratory abnormalities, significant bone disease, and/or vascular calcification that define chronic kidney disease–mineral and bone disorder (CKD-MBD) **(1).** Even with careful monitoring of these markers, patients with ESRD on maintained hemodialysis have poorer health outcomes related to mineral and bone disorder (MBD) such as the increased risk of developing cardiovascular disease and increased fracture risk **(1).** While obtaining abdominal radiographs, computed tomography-based imaging, or dual-energy X-ray absorptiometry scans or performing a bone biopsy to help evaluate calcification and bone mineral density (BMD) status, the strength of recommendation of these practices is low **(2)**.

Osteopontin (OPN) is a glycol-phosphoprotein found in bone, acute and chronic inflammatory cells, smooth muscle, epithelial and endothelial cells, neurons and fetal renal tissue and is expressed in the thick ascending limb of the loop of Henle. Some of its functions include increasing macrophage and T-cell counts, the perpetuation of inflammation, wound healing, tumor development and progression, roles in diabetes, and possible roles in regulating nephrolithiasis and nephrogenesis **(3).**

OPN was also found to promote angiogenesis, encourage growth and invasion of renal cancer, impact the development of lupus nephritis in patients with systemic lupus erythematosus, and potentially be useful as a marker of acute allograft rejection in kidney transplants **(4).** Local increases in OPN in vessel walls have been linked to atherosclerotic plaque formation, inflammation within arteries, and smooth muscle mineralization **(5).** The function of OPN in bone is defined by its ability to anchor osteoclasts via the $\alpha_v\beta_3$ integrin**. (6).**

PATIENTS AND METHODS

Study design and population:

A case-control study was conducted at Internal Medicine Department, Zagazig University Hospitals and Nephrology Department, Theodor Bilharz Research Institute from February 2021 to August 2021 on end-stage renal disease patients on regular hemodialysis and apparently healthy subjects. Demographic information was collected. The enrolled number of the study was 160 participants.

The population of the study:

This study included 160 subjects with different age groups and of both sexes. Participants were classified into two groups: Control group included 80 healthy subjects of both sexes with a mean age of 49.66 ± 19.96 years old, and patients' group that included 80 patients of both sexes with a mean age of 54.09 ± 15.6

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Inclusion Criteria:

Male and female aged > 18 years old, patients with CKD stage 5 (on hemodialysis), and control group of similar age, sex and distribution to cases of ESRD on dialysis

Exclusion Criteria:

Previous treatment with immunosuppressive drugs, active inflammatory diseases, acute infections, chronic or acute liver diseases, and any malignancy or history of treatment with chemotherapy or radiotherapy.

All studied groups were subjected to the following: Full History taking (history of DM, HTN…), Demographic characteristics including (age, sex and BMI), and laboratory investigations. Routine investigations: Complete blood picture was assayed on Sysmex Xn2000 (Japan), serum calcium, phosphorus, alkaline phosphatase, kidney function tests (blood urea and serum creatinine), liver function tests, total cholesterol and triglycerides all of these were assayed on full automated Cobas 8000, Roche diagnostics(Germany), PTH was assayed on full automated Cobas 6000, Roche diagnostics (Germany) and e-GFR using MDRD formula.

Special investigations:

Assay principle:

Serum OPN (was determined using an enzyme-linked immunosorbent assay-ELISA kit following the manufacturer's protocols). The kit was obtained from Bioassay Technology Laboratory(China).The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human osteopontin (OPN) in samples. The plate has been pre-coated with a human OPN antibody.

OPN present in the sample is added and binds to antibodies coated on the wells. And then, a biotinylated human OPN antibody was added and bound to OPN in the sample. Then Streptavidin-HRP was added and bound to the Biotinylated OPN antibody. After incubation, unbound Streptavidin-HRP was washed away during a washing step. The substrate solution was then added, and color develops in proportion to human OPN. The reaction was terminated by adding an acidic stop solution, and absorbance is measured at 450 nm.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis:

Data analysis was performed using SPSS (Statistical Package for the Social Sciences) version 20. Quantitative variables were expressed using their means and standard deviations. Categorical variables were described using their absolute frequencies and were compared using chi-square test. For ordinal binary data, chi-square for trend test was used. Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric tests.

The independent sample t-test (for normally distributed data) and the Mann Whitney test (for not normally distributed data) were used to compare quantitative data between two groups. Spearman rank correlation coefficient was used to measure the strength and association of correlation between two continuous variables. Linear regression analysis was used to determine independent factors associated with certain dependent factors. ROC curve was used to determine the best cutoff of certain quantitative parameters in diagnosing certain health problems. The level of statistical significance was set at $P \leq 0.05$. The highly significant difference was present if $p \leq 0.001$.

RESULTS

There was a statistically non-significant difference between the studied groups regarding age and gender. On the other hand, there was a statistically significant difference between them regarding BMI (higher in the control group) and smoking history (significantly lower in the case group). There was a statistically significant difference between the studied groups regarding comorbid diabetes or hypertension (no one within the control group had comorbid DM or hypertension) as shown in table (1).

Table (1): Comparison between the studied groups regarding demographic data and comorbidities

	Groups		Test	
	Case group N=80 (%)	**Control group N=80 (%)**	**χ^2/t**	**p**
Gender:				
Female	36 (45.0)	47 (58.8)	3.029	0.082
Male	44 (55.0)	33 (41.2)		
Age (years)				
Mean ± SD	54.09 ± 15.6	49.66 ± 19.96	1.562	0.120
Smoking:				
No	74 (92.5)	67(83.8)	9.536	0.002*
Yes	6 (7.5)	7 (16.2)		
BMI				
Mean ± SD	23.72 ± 4.25	27.32 ± 3.54	-5.815	<0.001**
Hypertension:				
No	8 (10.0)	80 (100)	130.909	<0.001**
Yes	72 (90.0)	0 (0)		
DM				
No	71 (88.8)	80 (100)	Fisher	0.003*
Yes	9 (11.2)	0 (0)		

X^2 Chi-square test t independent sample t-test **p≤0.001 is statistically highly significant

There was a statistically significant difference between the studied groups regarding hemoglobin (lower in the case group) and serum triglycerides (higher in the case group). On the other hand, there was a statistically non-significant difference regarding total cholesterol. There was a statistically significant difference between the studied groups regarding total calcium (lower in case group), phosphorus, and parathyroid hormone (both higher in the case group). On the other hand, there was a statistically non-significant difference between them regarding serum alkaline phosphatase (table 2).

Table (2): Comparison between the studied groups regarding hemoglobin, lipid profile, calcium, phosphorus, PTH and alkaline phosphatase (bone markers).

	Groups		Test	
	Case group	**Control group**	**t**	**p**
	Mean ± SD	**Mean ± SD**		
Hemoglobin (g/dL)	9.49 ± 1.56	12.32 ± 1.56	-11.495	<0.001**
T. cholesterol (mg/dL)	173.46 ± 5.42	163.73± 8.68	1.354	0.178
Triglycerides (mg/dL)	165.03 ± 9.52	106.85± 3.75	5.478	<0.001**
T. calcium (mg/dL)	7.38 ± 1.8	9.23 ± 0.81	-8.372	<0.001**
Phosphorus (mg/dL)	5.35 ± 1.89	3.3 ± 0.67	9.137	<0.001**
ALP U/L	7.00±1.36	83.3±3.85	-0.864	0.388
PTH (pg/ mL)	380.61±32.85	31±5.85	-9.209	<0.001**

t independent sample t-test **p≤0.001 is statistically highly significant

Table (3) showed that there was a statistically significant difference between the studied groups regarding serum urea, serum creatinine (all were lower in the control group), and eGFR (higher in the control group). There was a statistically non-significant difference between the studied groups regarding serum ALT and AST.

Table (3): Comparison between the studied groups regarding kidney and liver function test

	Groups		Test	
	Case group Mean ± SD	**Control group Mean ± SD**	**t**	**p**
S. urea (mg/dL)	130.95 ± 34.29	26.79 ± 5.35	26.57	<0.001**
S.Creatinine (mg/dL)	7.56 ± 1.3	0.83 ± 0.14	46.108	<0.001**
eGFR	6.78 ± 1.51	101.93 ± 7.81	-107.0	<0.001**
ALT (U/L)	20.66 ± 3.45	18.66 ± 4.26	1.842	0.067
AST (U/L)	20.1 ± 4.65	18.54 ± 4.29	2.319	0.161

t independent sample t-test **p≤0.001 is statistically highly significant

There was a statistically significant difference between the studied groups regarding osteopontin level, significantly was higher in the case group (Table 4).

Table (4): Comparison between the studied groups regarding Osteopontin level

	Groups		Test	
Osteopontin ng/mL	**Case group N=80**	**Control group N=80**	**Z**	**p**
Median	102.869	2.477	-10.802	<0.001**

Z Mann Whitney test *p < 0.05 is statistically significant

Table (5) showed that there was a statistically significant positive correlation between serum osteopontin level and each of hemoglobin, serum phosphorus, serum alkaline phosphatase and serum parathyroid hormone. On the other hand, there was a non-significant correlation between it and either age, BMI, urea, creatinine, eGFR, calcium, triglycerides, cholesterol, AST and ALT or total cholesterol.

Table (5): Correlation between osteopontin and the studied parameters

r	p
0.038	0.74
0.049	0.668
0.244	0.029*
-0.165	0.144
0	0.997
0.118	0.296
-0.096	0.397
0.244	0.029*
-0.091	0.422
-0.04	0.724
0.05	0.662
0.072	0.527
0.297	0.008*
0.306	0.006*

*p<0.05 is statistically significant r Spearman rank correlation coefficient

On doing linear stepwise regression analysis of factors significantly correlated to serum osteopontin, only serum PTH (unstandardized β=0.045, p<0.001) and hemoglobin level (unstandardized β=11.046, p=0.001), while serum alkaline phosphatase (unstandardized β=0.337, p > 0.05) was non-significantly associated with it (Table 6).

Table (6): Linear stepwise regression analysis of factors significantly correlated to serum osteopontin

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval	
	β	**Std. Error**	**β**			**Lower Bound**	**Upper Bound**
PTH (pg/ml)	0.045	0.012	0.391	3.895	<0.001**	.022	.068
Hemoglobin (g/dl)	11.046	2.115	0.351	3.546	0.001**	4.841	17.251
Alkaline phosphatase (U/L)	0.337	0.038	0.245	2.446	0.063	0.063	0.612

**p≤0.001 is statistically highly significant

The best cutoff of serum osteopontin in predicting chronic kidney disease was ≥31.1 with the area under curve 0.995 with the sensitivity of 98.8%, specificity 95%, positive predictive value 95.2%, negative predictive value 98.7% and accuracy of 96.9% (p<0.05) as shown in table (7) & figure (1).

Table (7): Performance of serum osteopontin in the prediction of chronic kidney disease among the studied participants

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≥31.1	**0.995**	**98.8**	**95**	**95.2**	**98.7**	**96.9**	**< 0.001****

***p<0.05 is statistically significant AUC area under curve PPV positive predictive value NPV negative predictive value.

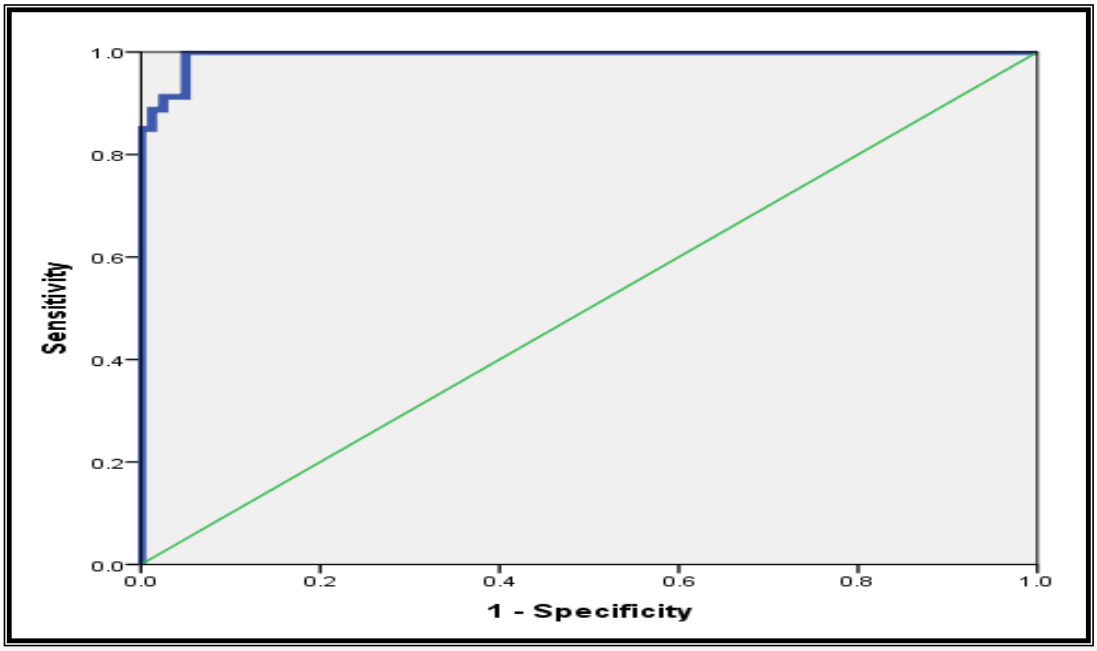

Figure (1): ROC curve showing performance of serum osteopontin in the prediction of chronic kidney disease among the studied participants.

DISCUSSION

In our study, there was statistically non-significant difference between the studied groups as regards age and gender. This disagrees with **Neugarten and Golestaneh** [8] who suggested that the preponderance of evidence indicates a protective role for the female sex in humans as well and that this effect is mediated by direct effects of sex hormones on renal biology. **Al-Wahsh *et al.*** [9] investigated the relationship between age and kidney failure in adults with category 4 chronic kidney disease and found that there is an inverse association between age and kidney failure, which is in contrast with our study.

In our study, there was a statistically highly significant difference between the studied groups as regard BMI (low in case group). This is in agreement with **Ahmadi *et al.*** [10] who found that HD patients had lower BMI and body weight after dialysis than predialysis. We also found that there was non-significant correlation between serum osteopontin and BMI. This is in line with the study of **Gordin *et al.*** [11] who reported that osteopontin did not correlate with BMI.

In our study, there was a statistically significant difference between the studied groups regarding the smoking history (P<0.001) (significantly lower in the case group). It is explained that most of the patients in this study stopped smoking after starting dialysis due to educational health of the hazards of smoking reported by many studies.

There is a close association between chronic kidney disease and hypertension, whether hypertension is a cause or a consequence of chronic kidney disease [12]**.** There was a statistically significant difference between the studied groups regarding comorbid diabetes or hypertension (no one within the control group had comorbid DM or hypertension because in the control group selection of subjects based on being healthy). This is in line with **Wang *et al.*** [13] who studied synergistic interactions of diabetes and hypertension in chronic kidney disease and reported that hypertension and DM interact synergistically to promote the progression of the renal injury. Anemia is a common and serious complication of CKD that presents during the early phase of the disease and worsens as the kidney function deteriorates [14]**.**

In our study, there was a statistically significant difference between both groups regarding hemoglobin level (P < 0.001), and there was a significant positive correlation between serum osteopontin and hemoglobin. This is in agreement with the study published by **Quaglia *et al.*** [15] who showed that high osteopontin levels were associated with anemia (p<0.001). This may further support the renal release of osteopontin since other authors showed that hypoxia upregulates osteopontin within renal tissue.

Although the kidney has no direct implications for lipoproteins metabolism, dyslipidemia is usually present in patients with CRF [16]**.** Hypertriglyceridemia is a common feature in patients with CKD, and this is due to an increased concentration of triglyceride-rich lipoproteins (TRL) (VLDL, chylomicrons and their remnants) [17]**.** This is in line with our study, where we found that there was a statistically significant difference between the studied groups regarding serum triglycerides (high in case group). On the other hand, our study showed a non-significant correlation between osteopontin level and serum triglyceride level. These findings are consistent with that reported by **Lorenzen *et al.*** [18] who did not find any association between osteopontin levels and serum triglycerides. In contrast **Robati *et al.*** [19] reported that plasma osteopontin level was positively associated with triglycerides (r = 0.35, P = 0.001). There was a statistically non-significant

difference between case and control groups regarding total cholesterol. This is consistent with other researchers, who have found levels of LDL cholesterol and total cholesterol are often within normal limits in CKD patients [20].

Secondary hyperparathyroidism (SHPT) is a common complication of chronic kidney disease (CKD), affecting over half of the patients with stage 3 or 4 CKD and 90% of those with end-stage renal disease (ESRD), based on the latest available data [21]. Impaired phosphate excretion and decreased synthesis of active 1, 25-dihydroxyvitamin D cause an elevated serum phosphate and decreased serum calcium, respectively, which directly stimulate parathyroid hormone (PTH) secretion [22]. This is in line with our results, which reviewed that there was a statistically significant difference between the studied groups regarding total calcium (lower in case group), while phosphorus and parathyroid hormone both were higher in case group. This is in agreement with previous researches who discussed abnormalities in minerals and bones in patients of chronic kidney disease.

There was a statistically non-significant difference between both groups regarding serum alkaline phosphatase in our study. This finding is in agreement with the study published by **Natikar *et al.*** [23] who found that there is an elevation in alkaline phosphatase levels in cases (50 patients of CKD) as compared to controls (50 apparently healthy subjects), but the increase was not statistically significant.

Chronic kidney disease severity varies from kidney damage with normal function to kidney failure (or end-stage renal disease), typically occurring when eGFR decreases to less than 15 ml/min per 1·73 m^2 [24]. Biochemical markers play an important role in accurate diagnosis, assessing risk, and adopting therapy to improve clinical outcomes. Serum analysis of renal function markers like urea, creatinine, uric acid, and electrolytes is routinely used [25]. In our study, there was a statistically significant difference between the studied groups regarding serum urea, serum creatinine (all were lower in the control group), and eGFR (high in the control group). This is logical because our study was carried out on two groups, case group included patients of chronic renal failure on regular dialysis and another group of apparently healthy subjects.

In our study, there was a statistically non-significant difference between the studied groups regarding serum AST and ALT. This is in contrast with **Ray *et al.*** [26] who reported that serum AST and ALT levels were significantly lower in CKD patients both without and with ESRD compared to controls.

Our results indicated a statistically significant difference between the studied groups regarding osteopontin level that was significantly higher in the case group. This is inconsistent with other researchers who found that plasma OPN concentration was proportional to the severity of renal function damage [27].

As regards the ROC curve analysis of the performance of serum osteopontin in the prediction of chronic kidney disease among the studied participants, The best cutoff of serum osteopontin in the prediction of chronic kidney disease was ≥31.1 with the area under curve of 0.995 with a sensitivity of 98.8%, specificity of 95%, positive predictive value of 95.2%, negative predictive value of 98.7% and accuracy of 96.9% ($p<0.05$)

Our study showed a positive correlation between serum osteopontin (OPN) level and the level of serum alkaline phosphatase, serum parathyroid hormone and serum phosphorus. The study of **Druck *et al.*** [28] showed similar results regarding serum alkaline phosphatase and serum parathyroid hormone, which displayed a positive correlation, but in contrast, this study reported no correlation found between OPN and serum phosphorus.

Regarding serum calcium; our study found that there was a non-significant correlation between OPN, and calcium and these findings are consistent with the previous study of **Atta** [29]. On the other hand, it does not agree with **Karakan *et al.*** [30], who reported that OPN positively correlated with serum calcium.

In our study, we noticed that there was a non-significant correlation between osteopontin and each of eGFR and creatinine. This is in contrast with **Lorenzen *et al.*** [18] who reported that circulating levels of osteopontin are closely related to glomerular filtration rate and that with chronic kidney disease, osteopontin plasma concentrations correlate inversely with GFR in patients with CKD (K/DOQI stages 1–5), as well as positively with other indicators of renal function such as serum creatinine.

Since there have been few studies on osteopontin in chronic renal failure and its relation to bone markers of CKD-MBD, measurement of serum osteopontin provides a suitable biomarker for early detection and monitoring the progression of CKD-MBD.

CONCLUSION:

From this study, we conclude that osteopontin may be considered as an early marker of chronic kidney disease. Routine monitoring of osteopontin levels in patients with ESRD on regular hemodialysis may help in early detection and prevention of chronic kidney disease-mineral bone disorders, AND Osteopontin levels may be a helpful marker for predicting mineral bone disease activity and prognosis in patients with ESRD on regular hemodialysis.

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