Hormonal and Mineral Imbalance Effect on Bone Resorption in Predialysis Iraqi Patients with Chronic Kidney Disease

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Abstract

Introduction: Chronic kidney disease mineral bone disorder is a metabolic bone disease present in almost all uremic patients. The aim of this research to indicate the stage of chronic kidney disease (CKD) that affect the bone metabolism that leading to the mineral and hormonal imbalance by studying the relationship among osteocalcin , glomerular filtration rate (GFR), parathyroid hormone, calcium and phosphorus levels in the blood.

Method: The study included 52 patients with predialysis chronic kidney disease stage 3-5 and 40 apparently healthy relatives accompanying the patients. Glomerular filtration rate (GFR) was calculated for each patient. Renal function tests, including serum levels of urea, creatinine, a biochemical marker of bone metabolism: osteocalcin (OSN), calcium, phosphorus, and parathyroid hormone (PTH), were measured for each participant.

Results: Serum urea and creatinine levels were significantly higher in CKD patients Than that of apparently healthy control. There is significantly higher serum parathyroid hormone, serum phosphorus, serum osteocalcin ($P<0.01$, $P<0.01$, $P<0.01$ respectively) in CKD patients than that of the healthy control group. While low serum calcium level in CKD patients as compared to the corresponding group ($P<0.01$).

Conclusion: Hyperphosphatemia and hypocalcemia in the end stage of predialysis CKD patients lead to increase parathyroid hormone secretion, which causes high bone turnover characterized by significantly high serum osteocalcin in these patients. Parathyroid hormone and osteocalcin were used as a biomarker for the development of bone and mineral disorders in predialysis CKD patients.

Keywords: *CKD, parathyroid hormone, osteocalcin, calcium, phosphorus*

Introduction

Chronic kidney disease (CKD) has become a public health problem. The definition of CKD was introduced by National Kidney Foundation in 2002 and later adopted by the international group Kidney Disease Improving Global Outcomes in 2004, a decrease in kidney function with a glomerular filtration rate (GFR) < 60 mL/min per 1.73 m^2 and/or kidney damage for 3 months or more [1] . Chronic kidney disease, mineral bone disorder (CKD-MBD) is a metabolic bone disease present in almost all uremic patients. With uremia, bone is relatively resistant to parathyroid hormone (PTH) action, such that the average level of PTH is required to maintain bone turnover [2]. Relative hypoparathyroidism is associated with low-turnover or a dynamic bone disease [3]; severe secondary hyperparathyroidism leads to high turnover bone disease [4]. Secondary hyperparathyroidism occurs in CKD produces an imbalance between osteoclast activity and osteoblast synthetic activity ,leading to enhanced bone breakdown at the end stage of renal disease [5].

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Osteocalcin(OSN) is a non-collagenous, vitamin K-dependent protein with 46–50 amino acid, produced by osteoblasts, it is a marker of bone formation [6]. High blood OSN levels are also in older adult humans, high levels in the blood are a predictor of lower bone density and a sign of fracture risk, including hip fractures and this because OSN levels can increase in the blood as a result of the breakdown of bone tissue [7]. The circulated OSN is removed by the kidney and liver[8], which increase in the blood when the renal function is declining. Patients with CKD show a progressive increase in serum OSN levels that closely corresponded with intact PTH and alkaline phosphatase levels. More fundamentally, such increases in serum OSN levels reflect the severity of the bone lesions [9].

Small increases in serum OSN were found in some patients at a severe stage, while a significant increase in blood OSN is shown in patients with end stage predialysis. In such patients, this elevation either due to decreased renal clearance of OSN or also reflected increased bone metabolism [10,11]. There is a negative correlation between glomerular filtration rate (GFR) and plasma osteocalcin levels in predialysis patients [12].

The aim of this research to indicate the stage of chronic kidney disease that affects the bone metabolism that leading to the mineral and hormonal imbalance by studying the relationship among OSN, GFR, PTH, calcium and phosphorus levels in the blood.

Method

The case-control study was conducted from March to Jun 2019, at the National Center of Teaching Laboratories of Medical City Institute, Baghdad, Iraq. Data of predialysis CKD Iraqi patient's attendant to Ghazi Alhariry hospital in Medical City in March 2019 for renal evaluation function were included in the study. The study was approved by the Ethics Committee of the University of Baghdad, Faculty of Pharmacy(UBCP-RECA-M62019A). 52 patients with predialysis chronic kidney disease stage 3-5 were enrolled, and 40 apparently healthy relatives accompanying the patients were selected. The purpose of the study and nature of all procedures were explained to participants, and informed approval was obtained before the commencement of the study. Patients were excluded if they had an acute infection, cancer, acute myocardial infarction, pulmonary edema, and patients on medication (steroid, bisphosphonates, calcium or vitamin D). The diagnosis of predialysis CKD patients was made by nephrologist based on the estimation of GFR together with renal function tests [table1]. GFR was calculated by the Modification of Diet in Renal Disease(MDRD) equation: 186 x (Creatinine/88.4)-1.154 x (Age)-0.203 x (0.742 if female) x (1.210 if black). Moderate reduction of GFR (30–59 mL/min/1.73 m^2) in stage 3, severe reduction of GFR (15–29 mL/min/1.73 m^2) in stage 4 preparation for renal replacement therapy and established kidney failure or end-stage renal disease (ESRD) (GFR <15 mL/min/1.73 m^2) in stage 5 requiring permanent renal replacement therapy (RRT).

The serum OSN was determined by Chemiluminescent enzyme immunoassay using Immulite 1000 autoanalyzer [13] (LKON1Siemens, USA). Serum urea nitrogen was measured by using urease/glutamate dehydrogenase coupled enzymatic technique (Dimension clinical chemistry System ,DF21 Siemens, USA). Serum creatinine was measured by using modified kinetic Jaffe technique (Dimension clinical chemistry System, DF33B Siemens, USA). Serum intact PTH was measured by using two-site chemiluminescent enzyme-labeled immunometric assay [14] (DPC Immulite 2000, Siemens, USA). Serum calcium and phosphorus levels were quantified by using Ca(DF23A), PO_4 (DF61A) Dimension Rxl Siemens autoanalyzer as per International Federation of Clinical Chemistry (IFCC) guidelines, modifications of calcium O-cresolphthalein complex one reaction (OCPC) and classical phosphomolybdate method, respectively [15,16].

Statistical Analysis

It was performed by using the SPSS Statistics version 20.0 The results were expressed as mean and standard deviation. Results were analyzed utilizing One-way ANOVA was used to determine the significance degree between parameters. The p-value ≤0.05 was considered significant. ROC curve was used to identify the validity of markers as an indicator of the disease.

Results

Serum levels of both urea and creatinine levels were significantly higher in CKD patients than apparently healthy control. These results were used together with the estimated GFR (< 60 mL/min per 1.73 m^2) to determine the stage of CKD in patients group [table 1] .

Table 1: Descriptive statistics between stages of CKD patients and healthy control.

Parameters	Stages	N	Mean	Std. Deviation	ANOVA test (P-value)
Age / Year	A.H. Control	40	44.61	11.505	P=0.00*
	3	12	63.60	2.510	
	4	20	58.95	5.781	
	5	20	49.85	10.713	
	Total	92			
Serum Urea	A.H. Control	40	29.67	3.029	P=0.00*
	3	12	52.20	9.445	
	4	20	67.55	11.651	
	5	20	191.85	71.582	
	Total	92			
Serum Creatinine	A.H. Control	40	0.805	0.123	P=0.00*
	3	12	1.740	0.288	
	4	20	2.760	0.454	
	5	20	9.230	4.123	
	Total	92			
GFR	A.H. Control	40	97.78	22.709	P=0.00*
	3	12	43.40	8.764	
	4	20	21.80	4.312	
	5	20	6.75	2.971	
	Total	92			
OSN	A.H. Control	40	6.12	7.012	P=0.00*
	3	12	11.90	8.389	
	4	20	26.21	16.848	
	5	20	41.04	22.372	
	Total	92			
Serum Calcium	A.H. Control	40	9.42	0.405	P=0.00*
	3	12	8.60	0.424	
	4	20	7.98	0.714	
	5	20	6.89	0.824	
	Total	92			
Serum phosphorous	A.H. Control	40	3.11	9.285	P=0.00*
	3	12	3.50	1.027	
	4	20	4.38	1.071	
	5	20	5.92	1.279	
	Total	92			
PTH	A.H. Control	40	22.33	6.791	P=0.00*
	3	12	40.60	14.311	
	4	20	60.35	19.906	
	5	20	94.70	23.939	
	Total	92			

*A.H : apparently healthy, CKD: chronic kidney disease, GFR: glomerular filtration rate, OSN: osteocalcin, PTH: parathyroid hormone Statistically significant at p <0. 05 , statistically highly significant at p-value ≤ 0.01 and non-significant at p>0.05

Curves of ROC studies confirmed that the blood urea and serum creatinine were a highly sensitive and specific diagnostic marker of CKD [figure1]. The sensitivity, specificity and cut-off point of hormonal and minerals for predialysis patients with CKD were estimated by ROC [table 2]

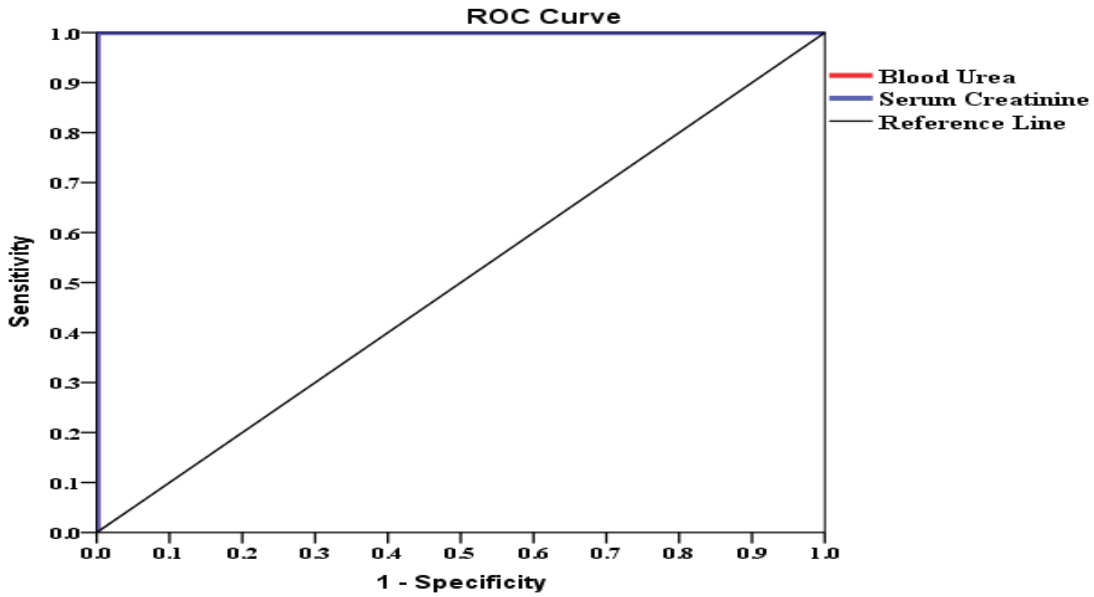

Figure 1: ROC for serum urea and creatinine levels

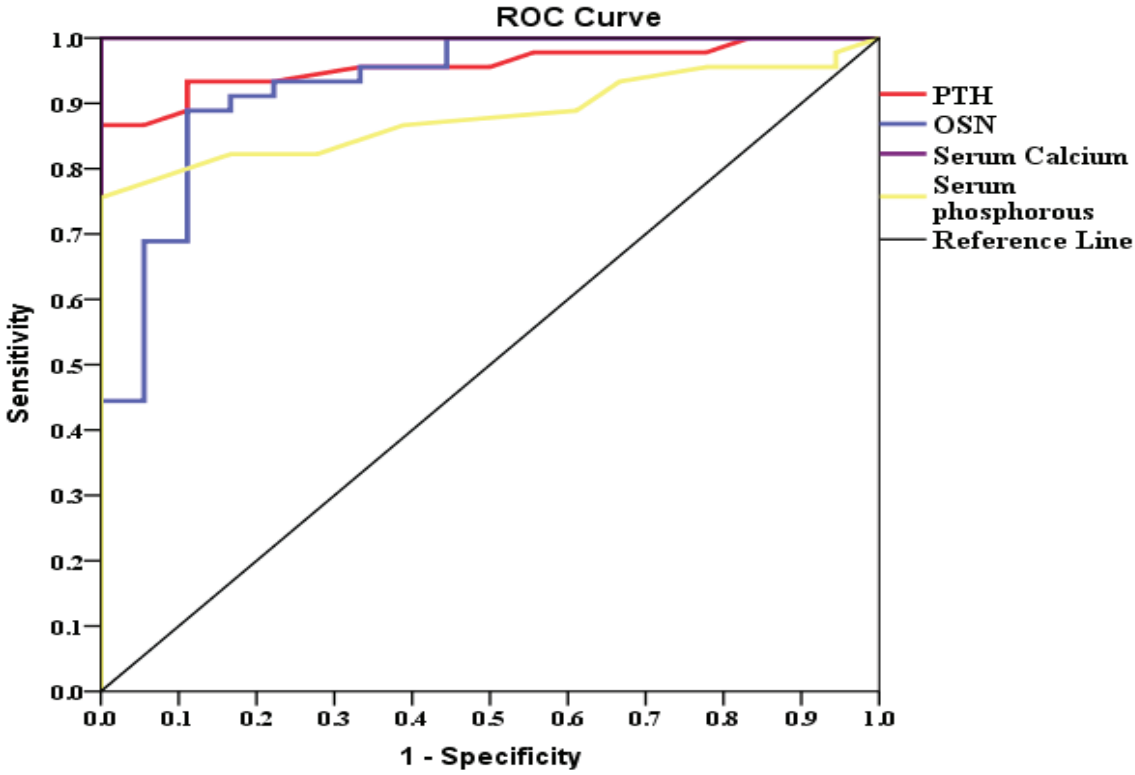

Figure 2: ROC curves for PTH, OSN, serum Calcium and serum phosphorus

Table 2: The cut-off point with sensitivity 100, specificity 100 and Area under the curve (AUC) 1.000 of laboratory results

Validity tests	Serum urea	Serum Creatinine	Serum Calcium	Serum phosphorous	OSN	PTH
Sensitivity	100%	100%	80%	86.7%	91.1%	95.6%
Specificity	100%	100%	100%	61.1%	77.8%	50%
Area Under the curve (AUC)	1	1	0.972	0.881	0.928	0.957
Cutoff value	> 38	> 1.2	< 8.5	> 3.1	> 6.6	> 21.5
P-value	0.00 HS	0.00 HS	0.00 HS	0.00 HS	0.00 HS	0.00 HS

HS=Highly significant difference (P<0.01)

Discussion

In the present study, the end stage of CKD patients has significantly higher serum concentration of the urea and creatinine as compared to stages 3-4 of CKD patients which is in agreement with other study[17]. This is due to the progressive reduction of GFR at the end stage of CKD patients. As the GFR decreases blood levels of both urea and creatinine are increased [18]. Significantly higher serum PTH levels at the end stage of CKD group among all studied patients groups, which is agree with the other findings which reported that the serum PTH was significantly higher in more advanced renal failure (stage 5 CKD), which confirms the relationship between severity of hyperparathyroidism and the degree of renal impairment [19,20]. Secondary hyperparathyroidism result from a decreased renal function, which is a common complication of CKD that leads to an overproduction of PTH caused by several changes that occur in bone and mineral metabolism because of decreased kidney function [21]. In this study, the CKD patients have hyperphosphatemia and hypocalcemia that leads to significant hyperparathyroidism when compared to healthy control patients with normal renal function.

At the end stage of CKD, when the remaining nephrons can no longer sufficiently excrete the phosphorus load, hyperphosphatemia is detected. The calcium and phosphorus form an insoluble complex in serum. This process may lead to extraskeletal calcification and potentially calciphylaxis or cardiac disease [22]. Retention of phosphorus also indirectly causes excessive production and secretion of PTH through lowering of ionized Ca^{2+} and by suppression of calcitriol production [19]. Vikrant S et al. was found increase serum PTH level in the end stage of CKD inversely correlated with GFR and serum calcium and positive correlation with serum phosphorus which is in agreement with this study [23]. Hyperphosphatemia is recognized as the primary initiator of the various cascades of the promoters of renal bone disease [24].

The present study shows increment serum osteocalcin levels associated with progressing stage of CKD up to higher concentration in the end stage CKD . Rix et al. who reported that patients with predialysis CKD had elevated serum levels of OSN with the more severe stage of CKD corresponding to the level of secondary hyperparathyroidism [25] as in agreement with this study.

In patients with impaired renal function plasma OSN levels are markedly elevated due to increased bone turnover and decreased renal elimination [26]. The effects of increased parathyroid hormone (PTH) resultant

resistance to adaptive stimulation of bone formation by parathyroid hormone, permits the effects of kidney injury to inhibit bone formation despite the development of secondary hyperparathyroidism [27].

Phosphate retention, hypocalcemia, and bony resistance to the action of PTH all these factors may contribute to overactivity of parathyroid gland to increase synthesis and secretion of PTH in end stage CKD. High levels of OSN in blood at the end stage of CKD occurs due to elevated PTH which stimulate bone demineralization that characterized by accelerated rates of bone absorption and resorption.

Conclusion

Hyperphosphatemia and hypocalcemia in the end stage of predialysis CKD patients lead to increase parathyroid hormone secretion, which causes high bone turnover characterized by significantly high serum osteocalcin in these patients. PTH and OSN were used as a biomarker for the development of bone and mineral disorders in predialysis CKD patients. It is recommended that attending physicians monitor and control biochemical parameters early in the development of CKD before the need for dialysis to protect the CKD patients from any complications that will result in response to PTH.

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