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Study of Serum Osteoprotegerin Level in Uremic Patients and Its Relation to Renal Bone Disease

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Abstract: *Introduction:* Bone disease associated with renal failure is termed renal osteodystrophy and is quite heterogeneous. Microscopic examination of a bone biopsy specimen is still considered the gold standard for diagnosis. Nevertheless, recent studies suggest that serum markers of bone formation and resorption may be of additional help in assessing bone turnover. Osteoprotegerin (OPG) and osteoprotegerin ligand (OPGL) constitute a complex mediator system involved in the regulation of the resorption process in bone. The present work aimed at studying the serum levels of osteoprotegerin and relation of osteoprotegerin to PTH and x-ray features of renal osteodystrophy has been studied. *Subjects and methods:* The present study was conducted on three groups Group I: 20 patients with chronic kidney disease(CKD) divided into two subgroups: Group Ia: 10 patients in stage 2 CKD (GFR of 60-89 ml/min) and stage 3 CKD (GFR of 30-59 ml/min).Group Ib: 10 patients in stage 4 CKD (GFR of 15-29 ml/min) and stage 5 CKD (GFR of <15 ml/min).Group II: 20 patients on maintenance hemodialysis for more than one year They were divided into two subgroups: Group IIa: 10 patients with intact PTH < 300 pg/ml. Group IIb: 10 patients with intact PTH > 300 pg/ml.Group III:10 age and sex matched healthy controls.Investigations will include measurements of Serum calcium, phosphorus, serum alkaline phosphatase, serum intact PTHand Assay of serum osteoprotegerin by enzyme linked immunosorbent assay (ELISA) technique. *Results:* Serum levels of OPG were higher in patients with CKD and patients on maintenance hemodialysis than healthy control. Serum levels of OPG were significantly higher in stage 4 and 5 CKD than in stage 2 and 3 CKD. *Conclusions:* Serum OPG level could be of help in the non-invasive diagnosis and monitoring of bone turn over state in patients with CKD.

Keywords: Osteoprotegerin, uremic pateints, diagnosing, Bone Disease

1. Introduction

Metabolic bone disease is a common complication of chronic kidney disease (CKD) and is part of a broad spectrum of disorders of mineral metabolism that occur in this clinical setting. Alterations in the control mechanisms for calcium and phosphorous homeostasis occur early in the course of CKD and progress as kidney function decreases. The disorders of bone have to be considered not only with regard to the bone itself but also with regard to the consequences of disturbed mineral metabolism at extra-skeletal sites, including the vasculature. [1] The National Kidney Foundation classifies renal osteodystrophy on the basis of:High turnover bone disease due to secondary hyperparathyroidism, the so called osteitis fibrosa cystic and Low turnover bone disease which is divided into Adynamic bone disease or Low turnover bone disease with mineralization defects, the so called osteomalacia.

In addition, other systemic processes that may affect the skeleton, such as the accumulation of β-2 microglobulin or the systemic effects of postmenopausal osteoporosis or steroid-induced osteoporosis, may complicate the picture.

1.1. Using Biochemical Markers in Renal Osteodystrophy

Bone disease associated with renal failure is termed renal osteodystrophy and is quite heterogeneous. [2]Microscopic examination of a bone biopsy specimen is still considered the gold standard for diagnosis. Measurement of serum intact

parathyroid hormone is an important guide to diagnosis and response to therapy. Nevertheless, recent studies suggest that serum markers of bone formation and resorption may be of additional help in assessing bone turnover. [3]

1.2. Issues in PTH Estimation

PTH level is used for years as a noninvasive biochemical method for classify and monitor renal bone disease. It was thought that the entire PTH molecule (1-84 aminoacid) was being estimated and thus they were called intact PTH (iPTH) assays.. However, it was realized that amino-terminally truncated fragments such as PTH (7-84) interfere with the estimation in the iPTH assays. The second generation radioimmunometric immunoassays that do not detect other PTH fragments lacking one or more aminoacid from the amino terminal are available. They can detect the entire PTH molecule (1-84) and are also called the biointact PTH (BiPTH) assay. The result of BiPTH is approximately 50 % of that for iPTH assays; In a study comparing the BiPTH and iPTH in predicting bone morphology, both assays were good in differentiating between the high and low turnover bone diseases, although BiPTH assay appear to provide a marginally better discrimination. [4]. Serum levels of PTH help in predicting the presence and severity of secondary hyperparathyroidism without correlating with the underlying bone disease. Although PTH is a good indicator of bone metabolism, the sensitivity and specificity to diagnose high turnover bone disease with levels < 500 ng/ml and ABD disease with levels < 100 ng/ml are inadequate.Levels of iPTH in dialysis patients more than 4 times normal and less than 2 times normal are associated with a greater frequency of high turnover (HTO) and low turnover (LTO) bone disease, respectively.

1.3. Role of Osteoprotegerin and Osteoprotegerin Ligand in Renal Bone Disease

Osteoprotegerin (OPG) and osteoprotegerin ligand (OPGL) constitute a complex mediator system involved in the regulation of the resorption process in bone, which is responsible for the homeostatic mechanism of bone turnover. Alterations in this system could be responsible for some metabolic bone diseases, like osteopetrosis and osteoporosis ,. [5,6]. A recent review [7] has showed the importance of this cytokine system which is able to control osteoclastic activity through the interplay of many factors, including PTH and calcitriol, that act mainly on the osteoblasts. It is known that OPG, which is secreted by osteoblasts, is able to block the osteoclastogenesis induced by OPGL, several studies have shown that PTH acts by enhancing the production of the osteoclastogenic factor OPGL and by inhibiting the synthesis of the soluble receptor OPG, which blocks the biological effect of OPGL.

OPG is expressed in many tissues apart from osteoblasts, including heart, bone marrow kidney, liver and spleen. [8] Its expression is regulated by factors that induce RANKL expression by osteoblasts. Many reports have supportedthat the RANKL/OPG ratio is a major determinant of bone mass. [9] An osteoprotective role for OPG in humans is supported by the report of homozygous deletions of 100 kilo bases of OPG in two patients with juvenile Paget's disease, an autosomal recessive disorder characterized by increased bone remodeling, osteopenia, and fractures. [10] It is also supported by the identification of an inactivating deletion in exon 3 of OPG in three siblings with idiopathic hyperphosphatasia, which is an autosomal recessive bone disease characterized by increased bone turnover associated with deformities of long bones, kyphosis, and acetabular protrusion in affected children. [11] A recent surprising finding is that OPG expression is regulated by Wnt/β-catenin signaling in osteoblasts, the same pathway that regulates osteoblastic bone formation. [12] Thus, bone mass is determined by the combined efforts of osteoblasts and osteoclasts, and is regulated in osteoblasts by two major signaling pathways: RANKL/RANK and Wnt/β-catenin.

OPG also appears to protect large blood vessels from medial calcification, detected by the observation of renal and aortic calcification occurring in OPG knockout mice [13] the absence of OPG in OPG/apolipoprotein E double knockout mice accelerates the calcific atherosclerosis, suggesting that OPG protects against this complication of atherosclerosis.[14] in human there is also an association between high levels of OPG in serum and cardiovascular disease, diabetes, and chronic renal failure [15] However, OPG in human setting does not appear to protect the skeleton against the increased bone resorption of secondary hyperparathyroidism mediated by PTH in patients with renal osteodystrophy or against vascular calcification. A possible explanation is that OPG in the serum of such patients is bound to plasma proteins and thus rendered inactive, further studies are needed to determine the significance of these observations.

2. Aim of the Work

The present work aimed at studying the serum levels of osteoprotegerin in patients with chronic kidney disease and patients on maintenance hemodialysis and find the relation of osteoprotegerin to PTH and x-ray features of renal osteodystrophy.

3. Patients and Methods

The present study was conducted on three groups:

Group I: 20 patients with CKD divided into two sub groups: *Group Ia:* 10 patients in stage 2 CKD (GFR of 60-89 ml/min) and stage 3 CKD (GFR of 30-59 ml/min).*Group Ib:* 10 patients in stage 4 CKD (GFR of 15-29 ml/min) and stage 5 CKD (GFR of <15 ml/min).

Group II: 20 patients on maintenance hemodialysis for more than one year (12 hours /week divided into 3 sessions using bicarbonate dialysate and polysulfone dialyzers).They were divided into two subgroups: *Group IIa:* 10 patients with intact PTH < 300 pg/ml. *Group IIb:* 10 patients with intact PTH > 300 pg/ml.

Group III: 10 age and sex matched healthy controls.

The patients were selected from the nephrology outpatient clinic and the dialysis unit of Alexandria Main university hospital and the dialysis unit of Al Mowassat University hospital. The controls were selected from those attending the outpatient clinics of Alexandria main university hospital. The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient and healthy control. All of the patients and control groups included in the study were subjected to the following: Assay of serum intact PTH [16] Serum calcium [17] and phosphorus. [18] Serum alkaline phosphatase.[19]Assay of serum osteoprotegerin by enzyme linked immunosorbent assay (ELISA) technique. [20] X- ray of bones of the hands and spine

4. Results

Table (1). *Comparison between the three studied groups according to demographic data.*

	Group I(n = 20)		Group II(n = 20)		Group III(n = 10)		Test of sig.
	No.	%	No.	%	No.	%	
Sex							
Male	12	60.0	14	70.0	8	80.0	p = 0.526
Female	8	40.0	6	30.0	2	20.0	
Age (years)							
Min. – Max.	38.0 – 75.0		23.0 – 65.0		30.0 – 67.0		$^{F}p = 0.068$
Mean ± SD	46.95 ± 9.32		40.25 ± 12.37		38.0 ± 11.58		

p: p value for comparing between the three studied groupsχ^2: Chi square test
F: F test f (ANOVA)

Table (2). *Descriptive analysis of group II according to the duration of hemodialysis.*

	Min. –Max.	Mean ± SD	Median
Duration of hemodialysis (years)	1.50 – 15.0	6.23 ± 4.03	5.50

Table (3). *Comparison between group I and group II according to the cause of CKD.*

Causes	Group I(n = 20)		Group II(n = 20)		Test of sig.
	No.	%	No.	%	
DM	9	45.0	2	10.0	$^{2}p = 0.013^{*}$
HTN	4	20.0	8	40.0	$^{2}p = 0.168$
Chronic GN	0	0.0	4	20.0	$^{FE}p = 0.106$
Obstructive nephropathy	3	15.0	2	10.0	$^{FE}p = 1.000$
Analgesic Nephropathy	2	10.0	0	0.0	$^{FE}p = 0.487$
Polycystic kidneys	1	5.0	0	0.0	$^{FE}p = 1.000$
Idiopathic	1	5.0	4	20.0	$^{FE}p = 0.342$
Total	20	100.0	20	100.0	

p: p value for comparing between the two studied groups
χ^2: Chi square testFE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table (4). *Comparison between the different studied groups according to intact PTH and osteoprotegerin.*

	Group I		Group II		Group III (n = 10)	P
	Ia(n = 10)	Ib(n = 10)	IIa(n = 10)	IIb(n = 10)		
Intact PTH (Pg/ml)						
Min. – Max.	34.30 – 754.0	13.30 – 610.70	56.50 – 256.0	713.3 – 1103.0	28.0 – 64.80	
Mean ± SD	195.10±219.72	201.72±200.78	165.48 ± 69.57	893.01±125.16	43.16±11.34	<0.001*
Median	129.75	109.0	174.40	889.65	44.50	
$^{MW}p_1$	0.035*	0.019*	<0.001*	<0.001*		
$^{MW}p_2$	0.796		<0.001*			
Osteoprotegerin (Pg/ml)						
Min. – Max.	61.40 – 403.40	113.90 – 902.0	100.0 – 698.10	76.60 – 908.30	38.80 – 69.0	
Mean ± SD	145.85±103.53	350.38±287.39	297.62±211.27	214.06±249.10	52.32± 13.01	<0.001*
Median	106.55	245.20	212.0	121.55	48.20	
$^{MW}p_1$	0.001*	<0.001*	<0.001*	<0.001*		
$^{MW}p_2$	0.009*		0.165			

p: p value for Kruskal Wallis test for comparing between the different studied groups$^{MW}p_1$: p value for Mann Whitney test for comparing between group III (control) and each other group(group Ia,Ib,IIa and IIb)$^{MW}p_2$: p value for Mann Whitney test for comparing between subgroups (Ia and Ib & IIa and IIb)
*: Statistically significant at $p \leq 0.05$.

There was a statistical significant difference between the studied groups as regards the intact PTH level (p=<0.001).Intact PTH was significantly higher in group Ia than Group III (p1=0.035) and significantly higher in group Ib than Group III (p1=0.019).

However there was no statistical significant difference between group Ia and group Ib as regards intact PTH level (p2=0.796).Intact PTH was significantly higher in group IIa than Group III (p1=<0.001) and was significantly higher in group IIb than Group III (p1=<0.001).

Also Intact PTH was significantly higher in group IIb than Group IIa (p1=<0.001).

There was a statistical significant difference between the studied groups as regards the OPG level (p=<0.001).

OPG was significantly higher in group Ia than Group III (p1=0.001) and significantly higher in group Ib than Group III (p1=<0.001).and significantly higher in group IIa than Group III (p1=<0.001).OPG was significantly higher in group IIb than Group III (p1=<0.001).

Also OPG was significantly higher in group Ib than group Ia (p2=0.009)

However there was no statistical significant difference between group IIa and IIb (p2=0.165).

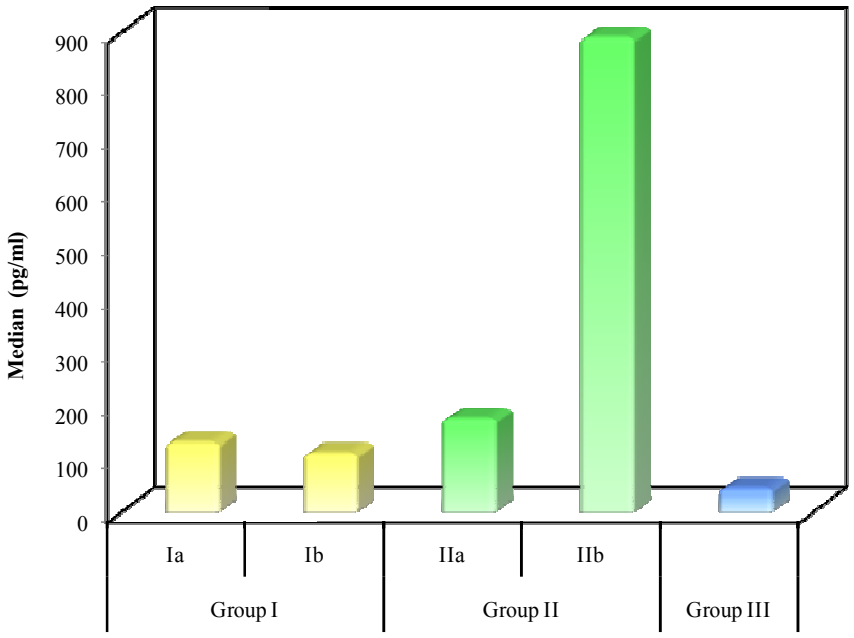

Figure (1). *Comparison between the different studied groups according to Intact PTH.*

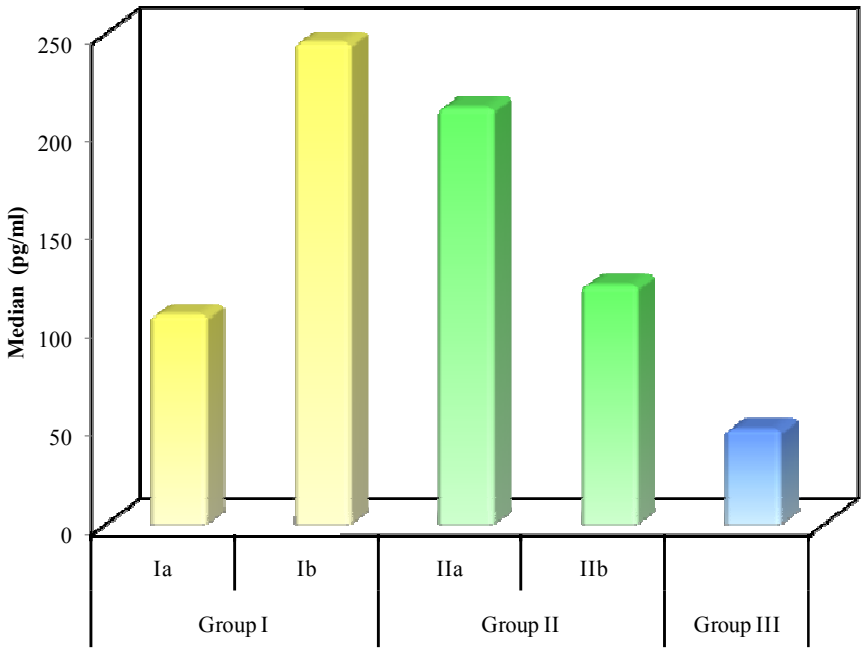

Figure (2). *Comparison between the different studied groups according to Osteoprotegerin.*

Table (5). *Correlation between osteoprotegerin and intact PTH in each studied group.*

Intact PTH (pg/ml)	Osteoprotegerin (pg/ml)	
	r_s	p
Group I	0.000	1.000
Group II	-0.141	0.552
Group III	0.085	0.815

r_s: Spearman coefficient

In group I: There was no correlation between serum OPG level and intact PTH (r=0.000, p=1.000).

In group II: There was an insignificant negative correlation between serum OPG level and intact PTH (r= -0.141, p=0.552).

In group III: There was an insignificant positive correlation between serum OPG level and intact PTH (r=0.085, p=0.815).

Subperiosteal resorption:In group I: No patient showed subperiosteal bone resorption. *In group II:* There was no statistical significant difference in the mean OPG serum level between the patients showingsubperiosteal bone resorption and those without (p=0.450).

Rugger jersy spine:In group I: There was no statistical significant difference in the mean OPG serum level between the patient showing rugger jersy spine and those without (p=0.435). *In group II:* There was no statistical significant difference in the mean OPG serum level between the patients showing rugger jersy spine and those without (p=0.529).

Osteopenia:In group I: There was no statistical significant difference in the mean OPG serum level between the patients showing osteopenia and those without (p=0.965).*In group II:*There was no statistical significant difference in the mean OPG serum level between the patients showing osteopenia and those without (p=0.909).

Looser mann's pseudofractures:In group I: No patient showed Looser mann's pseudofractures.*In group II:*There was no statistical significant difference in the mean OPG serum level between the patients showing Looser mann's pseudofractures and those without (p=0.099).

Table (6). *Correlation between osteoprotegerin and x-ray findings in group I and group II.*

		N	Osteoprotegerin			P
			Min. – Max.	Mean ± SD	Median	
Group I	Subperiosteal resorption	0	-	-	-	-
	Rugger jersy spine	1	246.80 – 246.80	246.80 ± -	246.80	0.435
	Osteopenia	5	76.70 – 860.40	293.88 ± 325.63	206.50	0.965
	Looser mann's pseudofractures	0	-	-	-	-
Group II	Subperiosteal resorption	4	100.10 – 204.30	149.53 ± 47.20	146.85	0.450
	Rugger jersy spine	2	100.10 – 204.30	152.20 ± 73.68	152.20	0.529
	Osteopenia	9	97.80 – 908.30	246.60 ± 252.93	171.60	0.909
	Looser mann's pseudofractures	1	908.30 – 908.30	908.30 ± -	908.30	0.099

p: p value for Mann Whitney test

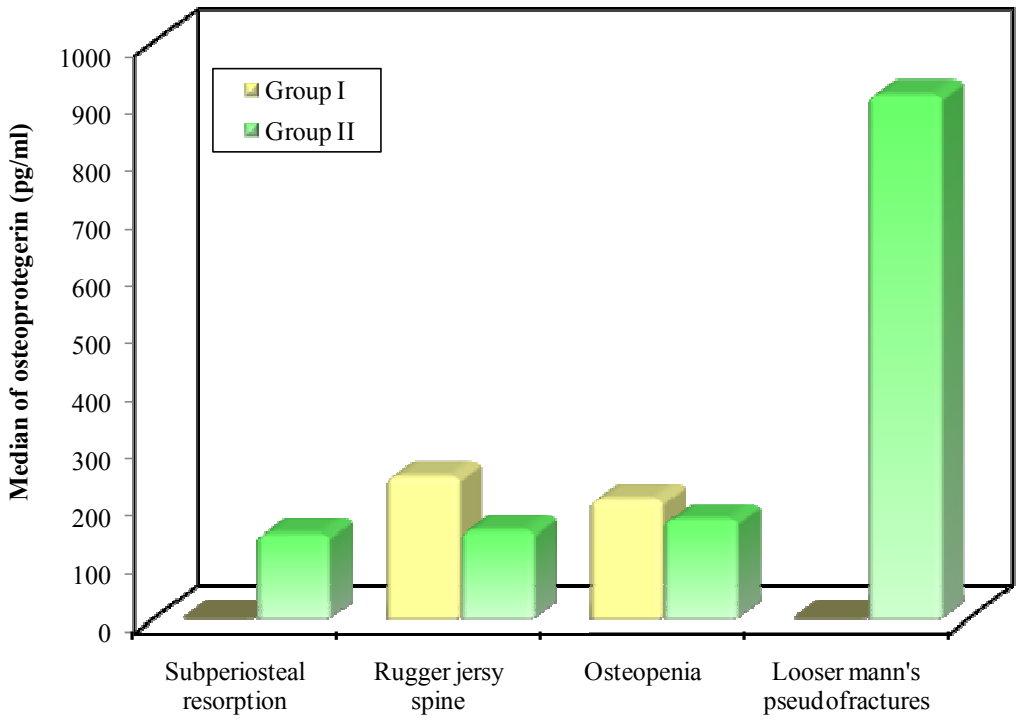

Figure (3). *Correlation between osteoprotegerin and x-ray findings in group I and group II.*

Table (7). *Correlation between osteoprotegerin and the duration of hemodialysis in group II.*

	Duration of hemodialysis	
	r_s	P
Osteoprotegerin (Pg/ml)	0.776	<0.001*

In group II: There was a significant positive correlation between serum levels of OPG and the duration of hemodialysis (r=0.776, p<0.001).

5. Discussion

In the present study, there was a statistical significant difference between the three studied groups as regards the OPG level (p=<0.001), OPG level was significantly high in all the subgroups of patients when compared to group III of the healthy control (p= <0.001).

These data coincides with the study of Vattikuti et al [21] which found that patients with renal failure have increased serum OPG concentration that is decreased after transplantation. Our data also coincides with that of Kazama et al [22] who reported elevated OPG levels in CKD patients and suggested that accumulated OPG in the circulation may be a uremic toxin that increases the skeletal resistance to PTH. Moreover, Nessim et al [23] studied serum levels of OPG in sixty CKD patients in stages 3, 4 and 5 and reported a strong negative association between OPG and eGFR and detected an increase in serum OPG with progression of CKD.

The previous results ofNessim et al [23] are compatible with our results, as in the present study, we found a statistical significant difference between subgroups Ia and Ib where OPG was significantly higher in subgroup Ib (p=0.009).

However, there was no statistical significant difference between subgroup IIa and IIb as regards serum OPG level (p=0.165) in the present study. These data is consistent with the study of Kazama et al [22] which showed that serum OPG levels in uremic patients were elevated and independent of their serum PTH levels, what suggested the circulating OPG to be an independent factor affecting bone metabolism in uremic patients.

In the present study, we didn't find any significant correlation between OPG and intact PTH in all the studied groups as follows; for group I, there was no any correlation between OPG and intact PTH (r=0.00, p=1.000), and in group II, there was an insignificant negative correlation between OPG and intact PTH (r= - 0.141, p=0.552) while in group III, OPG correlated weakly with intact PTH (r= 0.085, p=0.815).

Celic et al [24] studied bone metabolism in CKD patients, both predialysed and hemodialysed as well as kidney transplant recipients. The study included 4 groups; group I (40 CKD patients in stage 3 and stage 4), group II (90 patients on maintenance hemodialysis) and group III (30 renal allograft recipients enrolled at the time of transplantation and followed up for 6 and 12 months post transplantation). These groups were compared with 40 ages and sex matched healthy individuals.

They demonstrated that serum levels of OPG and intact PTH are significantly higher in the predialysed and hemodialysed group compared to the control group. Predialysis and hemodialysis groups have similar concentrations of OPG (p=0.004), while significantly higher levels of intact PTH are found in the hemodialysis groups (p=0.008).During first year post transplantation, serum levels of OPG were normal and similar to those of the control group. Serum levels of intact PTH also declined significantly 1 year post transplantation where 80% of the allograft recipients had values between 50-150 pg/ml and 20% of them attained values less than 50 pg/ml. Also in their study, they detected a significant negative correlation between OPG and intact PTH in all the studied groups (r= - 0.382, p=0.003).

When comparing the results of the afore mentioned study with the current one, we find that it mismatches our correlation between OPG and intact PTH in group I (the CKD group) and group III (the control group) and this may be attributed to the smaller sample size in our study, while it poorly coincides with our correlation in group II (the hemodialysis group).

Coen et al [25] in their study which was conducted on 39 patients on maintenance hemodialysis to evaluate the serum levels of OPG in different bone histological patterns of chronic renal failure and to establish a possible relationship between its serum levels and those of PTH as well as histomorphometric and histodynamic parameters, provided valuable information on the role of OPG on renal osteodystrophy.

They found that serum OPG levels were on average above the normal range. They were lower in adynamic bone disease patients than in patients with high bone turn over or mixed osteodystrophy.A significant negative correlation was found between serum OPG and PTH levels in high bone turn over and mixed osteodystrophy patients with PTH values ≤ 1000 pg/ml. This negative correlation was not observed in three patients with PTH >1000 pg/ml in whom OPG levels were relatively elevated suggesting an increased production of OPG when the osteoblastic population is maximally stimulated by PTH.

An additional observation in Coen et al [25] study was the finding of a significant difference in OPG serum levels between patients with adynamic bone disease and those with high bone turn over and mixed osteodystrophy at PTH levels of ≤ 300 pg/ ml. It is known that PTH levels ranging from 100 to 300 pg/ ml define an area of uncertain bone turn over in chronic renal failure. At PTH serum levels of ≤ 300 pg/ ml, the average value of OPG was significantly lower in patients with adynamic bone disease than in patients with high bone turn over and mixed osteodystrophy. This finding might suggest that serum OPG assays might be useful for distinguishing between low turnover bone disease and high turnover renal osteodystrophy, at least in the range of PTH values where a clinical diagnosis is in doubt.

The results of Coen et al [25] study differ from our

correlation in group II (the hemodialysis group) as they reported a strong negative correlation between serum levels of OPG and intact PTH which is not observed beyond PTH values > 1000 pg/ml. The difference in our results and Coen et al [25] study may be due to the smaller sample size in the present study.

Concerning the effect of hemodialysis, we observed a significant positive correlation between serum levels of OPG and the duration of hemodialysis in group II ($r=0.776$, $p<0.001$).

This result of our study matches that of Doumouchtsis et al [26] and Doi et al [27] who reported dependent increase in serum levels of OPG with the duration of hemodialysis and suggested that OPG accumulates in the serum of hemodialysis patients

In the present study, there was no statistical significant difference between the mean serum OPG level in patients showing x-ray findings of renal osteodystrophy (subperiosteal erosion, rugger jersy spine, osteopenia and looser mann's pseudofracture) and the mean serum OPG level in patients lacking these findings in both group I and II.

To our knowledge, data about the relationship between serum level of OPG and x-ray finding of renal osteodystrophy are limited.

Finally, from our study and all the previously discussed studies, we observed the role of OPG in the development of renal bone disease. Serum OPG level could be of help in the non-invasive diagnosis and monitoring of bone turn over state in patients with CKD.

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