Biochemical evaluation of renal osteodystrophy in uraemic patients

Maha H. Mohamed[a], Magda S. Mahmoud[a], Mohamed D.E. Abd El-Maksoud[a], Tamer E. Mosa[a], Adel A. Ali[b]

[a]Department of Biochemistry, National Research Centre, [b]Department of Internal Medicine, Faculty of Medicine, Alazhr University, Cairo, Egypt

Correspondence to Mohamed D.E. Abd El-Maksoud, PhD, Biochemistry Department, National Research Centre, Cairo, Egypt
Tel: +20 111 793 2321;
e-mail: dr_mohameddiaa1965@yahoo.com

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Background/aim
Renal osteodystrophy is a multifactorial disorder of bone remodelling that develops in patients with chronic renal failure (CRF). Biochemical markers of bone turnover have been proposed for the noninvasive diagnosis of renal osteodystrophy. The purpose of this study was to evaluate intact parathyroid hormone (iPTH) and some markers of bone disease in predialysis (preD) and haemodialysis (HD) CRF patients and correlate them with bone mineral density (BMD).

Patients and methods
Several biochemical markers were measured in the serum of 74 CRF patients (38 preD patients and 36 patients on regular HD). In addition, 30 healthy volunteers were included as controls. BMD of all patients was measured by means of calcaneal ultrasonography.

Results
BMD was measured by means of ultrasound. BMD was significantly decreased in both patient groups when compared with controls. Also, it was significantly lower in patients with osteoporosis than in those with osteopenia. iPTH, total alkaline phosphatase (ALP) and osteocalcin (OC) levels were significantly elevated in both patient groups when compared with controls. Ionized calcium (Ca^{2+}), free carnitine and insulin-like growth factor-1 (IGF-1) levels were significantly decreased in patients compared with controls. There was a significant inverse correlation of BMD with iPTH, ALP and OC and a significant positive correlation with Ca^{2+} and IGF-1 in HD patients. PreD patients showed significant inverse correlation of BMD with iPTH and ALP and significant positive correlation with Ca^{2+}.

Conclusion
The results of the present study suggested that ultrasound is a useful method for evaluating BMD and provides information about diverse regional skeletal changes in CRF patients. iPTH, ALP, OC and Ca^{2+} can predict renal osteodystrophy in preD and HD CRF patients. PreD and HD CRF patients often have low serum concentrations of free carnitine and IGF-1.

Keywords:
biochemical markers, calcaneal ultrasound, free carnitine, insulin-like growth factor-1, renal osteodystrophy

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Introduction

Renal osteodystrophy (ROD) is a well-known bone pathology appearing in patients with chronic renal failure (CRF), especially in predialysis (preD) and dialysis patients, and manifests in three different forms: high-turnover ROD, related to secondary hyperparathyroidism; low-turnover ROD and mixed ROD [1]. Bone histology is the gold standard for the diagnosis of ROD especially for the distinction between high-turnover and low-turnover bone disease but is an expensive and invasive procedure accompanied by technical difficulties [2]. For this reason, specific and sensitive serum biochemical markers are required for monitoring bone turnover in uraemic patients. The ideal biochemical marker of bone turnover should be unique to bone and should reflect total skeletal activity [3]. The serum bone markers are enzymes of the bone cells, components of the bone matrix or regulating hormones [2].

The parathyroid hormone (PTH) is a major regulator of bone turnover and skeletal cellular activity and its measurement in serum or plasma has been widely used. PTH in the serum occurs as an intact hormone and as many different PTH fragments [4]. Alkaline phosphatase (ALP) is a glycosylated protein produced by different organs such as the liver, bone, kidney, intestine and placenta [5]. One single gene codes a group of ALPs that are produced by the liver, bone and kidney and their respective isoenzymes differ only by post-transcriptional glycosylation [6].

Urena *et al.* [7] showed that the blood osteocalcin (OC) concentration is used as one of the sensitive markers of bone formation and reflects the underlying bone histology in ROD. They also reported that the plasma levels of OC demonstrated good sensitivity, allowing the distinction between patients with hyperparathyroidism and those with normal or low bone turnover. However, it

has low diagnostic sensitivity in discriminating between patients with adynamic bone disease and those with normal bone turnover. Bervoets *et al.* [8] demonstrated that OC, ALP, bone alkaline phosphatase (bALP) and serum calcium levels are useful in the diagnosis of adynamic bone disease, normal bone and osteomalacia in preD patients with end-stage renal failure.

Carnitine plays a critical role in energy production. It transports long-chain fatty acids into the mitochondria so that they can be oxidized (burned) to produce energy. It also transports the toxic compounds generated out of this cellular organelle to prevent their accumulation. Given these key functions, carnitine is concentrated in tissues like skeletal and cardiac muscle, which utilize fatty acids as a dietary fuel [9].

In the blood, ~97% of the insulin-like growth factor (IGF) is bound to six IGF-binding proteins (IGFBP-1 to IGFBP-6), with the remaining IGF-1 either in a bioactive-free fraction (~1%) or in an easily dissociable form [10]. Most IGF-1 in the circulation is bound in a 150-kDa complex of IGF-1, IGFBP-3 and a third protein, the acid-labile subunit. This ternary IGF complex is a storage form of IGF in blood and has a half-life of several hours. The binding of IGF-1 to IGFBP limits the bioactivity of IGF-1, as bound IGF-1 probably cannot activate the IGF-1 receptor [11].

In this study we investigated the value of biochemical markers of bone turnover in the diagnosis of ROD in preD and haemodialysis (HD) CRF patients.

Patients and methods

This study was approved by the Medical Ethical Committee of Al-Azhar University, Faculty of Medicine, and informed written consent was obtained from each participant before being enrolled in the study. Seventy-four CRF patients, 38 preD (before the first dialysis) patients and 36 patients on regular HD admitted to Al-Hussien University Hospital (46 men and 28 women; mean age 48.2 years, range 25–53 years) were included in this study. In addition, 30 healthy volunteers of matched age and sex were included and considered as controls. All individuals were subjected to a full medical history and a thorough clinical examination. CRF patients on corticosteroid treatment and those with diabetes mellitus, hepatic affection or hyperthyroidism were excluded. HD patients were divided into two groups (the osteopenia group and the osteoporosis group) according to bone mineral density (BMD). Osteopenia is a condition in which BMD is lower than normal. It is considered by many doctors to be a precursor to osteoporosis. Osteoporosis, however, is a progressive bone disease that is characterized by a decrease in bone mass and density that can lead to an increased risk for fracture. BMD was measured using ultrasound; osteopenia is defined by the WHO as a value of BMD (expressed as *T*-score) between -1 and -2.5. Osteoporosis was defined as a BMD value below -2.5 (i.e. *T*-score: normal, >-1; osteopenia, -1 to -2.5; osteoporosis, <-2.5) [12].

Sampling

Fasting 5 ml venous blood was obtained from all included individuals. One portion of the blood was collected in EDTA tubes and plasma was separated for determination of glucose. The other portion of the blood was collected in EDTA-free tubes, allowed to clot and centrifuged at 5000 rpm for 10 min. Serum was stored at -70°C until analysis.

Analytical methods

Peripheral haemogram, liver function test and kidney function test were carried out in all patients. Plasma glucose level was estimated by the GOD-PAP enzymatic colorimetric method using a Biomerieux test kit [13]. Serum K^+ and Na^+ concentrations were measured with a flame photometer (model PFP7, Jenway). Levels of intact parathyroid hormone (iPTH) were determined using Immulite (chemiluminescent enzyme-labelled immunometric) assay kit (Diagnostic Products Corporation, Los Angeles, USA). The enzyme activity of ALP was estimated colorimetrically using phenyl phosphate as a substrate [14]. Human OC levels were determined using an immunoenzymatic assay kit (Biosource Europe SA, Nivelles, Belgium) for the quantitative determination of intact human OC in serum. Serum ionized calcium (Ca^{2+}) concentration was determined with an atomic absorption spectrophotometer (AA-630-02; Shimadu, Japan) using an air-acetylene flame and hollow cathode lamp under the following parameters: lamp current, 10 mA; wavelength, 422.7 nm; and calcium standard, 5 ppm. Levels of IGF-1 in serum were determined using the ELISA kit (Biosource Europe SA). As IGFBP-3 interferes in the determination of IGF-1, it was essential to include an extraction step using ethanol acid solution (87% ethanol+12.5% HCl 2 N) in which IGF-1 was separated from its binding proteins [15]. Serum-free carnitine (FC) levels were determined according to the method of Wan and Hubbard [15] using a random-access chemistry analyser.

Statistical analysis

SPSS program (version 9.0; SPSS Inc., Chicago, Illinois, USA) was used for analysis of data. Statistical evaluation was carried out with Pearson's (*r*) correlation

coefficient, one-way analysis of variance and the Kruskal–Wallis test. Comparison between the two groups was made using the Mann–Whitney *U*-test. Data are expressed as mean ± SE. Significance was defined at *P* value less than 0.05.

Results

The demographic and laboratory parameters for renal function in preD and HD patients are shown in Table 1. There was a statistically significant reduction in serum urea, creatinine, K^+ ($P > 0.001$) and Na^+ ($P > 0.05$) concentrations in HD patients compared with preD patients, whereas haemoglobin concentration showed a statistically significant increase in the HD group compared with the preD group ($P > 0.001$).

CRF patients showed a statistically significant elevation ($P > 0.05$) in ALP, iPTH and OC levels, whereas serum levels of Ca^{2+}, IGF-1 and FC were significantly decreased ($P > 0.05$) when compared with the control group (Table 2).

HD and preD patients showed statistically significantly lower BMD compared with control participants, and the HD group showed statistically significantly lower BMD compared with the preD group. HD patients demonstrated a further statistically significant increase in ALP, iPTH and OC levels when compared with either preD patients or control participants and these levels were higher in preD patients when compared with controls. Also, there was a statistically significant decrease in Ca^{2+}, FC and IGF-1 levels in the HD group when compared with the preD group and controls and in the preD group when compared with controls (Table 3).

The HD group ($n = 36$) showed significant changes in parameters when it was divided into the osteopenia subgroup ($n = 22$) and the osteoporosis subgroup ($n = 14$) according to the results of BMD. The osteoporosis subgroup had statistically significantly lower BMD compared with the osteopenic subgroup ($P > 0.001$). There was a statistically significant increment in ALP, iPTH and OC in osteoporosis patients compared with osteopenic patients ($P > 0.01$). Also, there was a statistically significant decrease in Ca^{2+} ($P > 0.01$) and IGF-1 ($P > 0.05$) in the osteoporosis subgroup compared with the osteopenic subgroup. Data showed that there were no significant differences in serum levels of FC between these two subgroups (Table 4).

The correlations among studied parameters in preD patients and HD patients are shown in Tables 5 and 6. Figures 1–3 show the role of FC in the pathogenesis of ROD by its negative correlation with both ALP and

Table 1 Demographic and laboratory parameters for renal function in predialysis and in haemodialysis patients

Variables	PreD ($n = 38$)	HD ($n = 36$)	*P*
Age (years)	47 ± 1.45	49 ± 1.82	NS
Sex (male/female)	22/16	23/13	NS
Urea (mg/dl)	420 ± 6.18	217.5 ± 17.54	<0.001
Creatinine (mg/dl)	16.1 ± 0.67	10.94 ± 0.75	<0.001
Hb (g/dl)	6.19 ± 0.44	9.41 ± o.46	<0.001
Na^+ (mmol/l)	155 ± 6.1	132 ± 1.4	<0.05
K^+ (mmol/l)	6.81 ± 0.15	5.65 ± 0.17	<0.001

All data are expressed as mean ± SE; Hb, haemoglobin; HD, haemodialysis; preD, predialysis.

Table 2 Biochemical parameters in chronic renal failure patients compared with controls

Parameters	Control ($n = 30$)	CRF patients ($n = 74$)	*P*
Ca^{2+} (mg/dl)	11.31 ± 0.33	8.63 ± 0.3	<0.05
Range	9–11.8	5.99–10	
ALP (U/l)	55.5 ± 5.66	155.7 ± 9.3	<0.05
Range	19.9–89.6	69–278	
iPTH (pg/ml)	44.5 ± 4.53	489.4 ± 53.4	<0.05
Range	17–77	36.4–1215	
OC (ng/ml)	14.4 ± 1.59	69.2 ± 6.21	<0.05
Range	7.8–28	8–116	
IGF-1 (ng/ml)	335.4 ± 13.98	211.3 ± 9.92	<0.05
Range	221.3–398.90	129–405	
FC (μm/l)	21.42–0.76	16.9 ± 0.498	<0.05
Range	18.3–26.7	9.8–28.7	
Na^+ (mmol/l)	141.6 ± 1.07	145.4 ± 2.95	NS
Range	135–147	120–190	
K^+ (mmol/l)	4.27 ± 0.13	6.3 ± 0.137	<0.05
Range	3.8–5	4.7–7.9	

All data are expressed as mean ± SE; ALP, alkaline phosphatase; CRF, chronic renal failure; FC, free carnitine; IGF-1, insulin-like growth factor-1; iPTH, intact parathyroid hormone; OC, osteocalcin.

Table 3 Markers of renal osteodystrophy, insulin-like growth factor-1 and free carnitine in the studied groups

Parameters	Control ($n = 30$)	PreD ($n = 38$)	HD ($n = 36$)
BMD	0.5 ± 0.3	-1.62 ± 0.32[a]	-2.55 ± 0.26[b,c]
Ca^{2+} (mg/dl)	11.31 ± 0.33	8.76 ± 0.32[a]	8.53 ± 0.31[b,c]
Range	9–11.8	7.72–9.83	5.99–10
ALP (U/l)	55.5 ± 5.66	108 ± 6.73[a]	178.3 ± 11.99[b,c]
Range	19.9–89.6	69–181	108–278
iPTH (pg/ml)	44.5 ± 4.53	281 ± 61.92[a]	713.3 ± 68.77[b,c]
Range	17–77	36.4–1103	88.9–1215
OC (ng/ml)	14.4 ± 1.59	51.11 ± 7.99[a]	80.23 ± 7.68[b,c]
Range	7.8–28	8–102	32.5–116
IGF-1 (ng/ml)	335.4 ± 13.98	233.5 ± 15.1[a]	208 ± 11.6[b,c]
Range	221.3–398.90	131–405	129–399
FC (μm/l)	21.42 ± 0.76	18.6 ± 0.69[a]	15.7 ± 0.59[b,c]
Range	18.3–26.7	10.88–26.3	9.8–28.7

All data are expressed as mean ± SE and range; ALP, alkaline phosphatase; BMD, bone mineral density; FC, free carnitine; HD, haemodialysis; IGF-1, insulin-like growth factor-1; iPTH, intact parathyroid hormone; OC, osteocalcin; preD, predialysis; [a]PreD vs. controls; [b]HD vs. controls; [c]PreD vs. HD.

iPTH and its positive correlation with IGF-1 among all studied CRF patients.

Discussion

Over the past one or two decades, the principal biochemical marker for diagnosis and classification and for monitoring therapy of bone turnover has been measured by a two-site immunometric technique called 'intact' PTH [4]. Although measurement of PTH is the mainstay of the assessment of bone turnover and thus the precise type of ROD, other biochemical markers may also be useful. Measurements of total ALP should be considered in conjunction with determinations of PTH [7]. Measurements of total ALP are complicated by the measurement of nonskeletal enzyme, and accordingly, in some cases, measurements of bone-specific ALP may be required. However, there are insufficient data available to recommend routine measurement of bone-specific ALP concentrations [4].

Table 4 Impact of haemodialysis on markers of renal osteodystrophy, insulin-like growth factor-1 and free carnitine in osteopenia and osteoporosis

Parameters	Osteopenia (n = 22)	Osteoporosis (n = 14)	P
BMD	-1.88 ± 0.16	-3.66 ± 0.34	<0.001
Ca^{2+} (mg/dl)	8.3 ± 0.3	6.86 ± 0.06	<0.01
ALP (U/l)	128 ± 10.88	151.9 ± 9.88	<0.01
iPTH (pg/ml)	519.8 ± 105.3	829.3 ± 110.2	<0.01
OC (ng/ml)	57.7 ± 8.21	91.43 ± 6.43	<0.01
IGF-1 (ng/ml)	238.8 ± 18.46	174.3 ± 19.44	<0.05
FC (μm/l)	14.75 ± 0.88	13.43 ± 0.85	NS

Data are expressed as mean ± SE; ALP, alkaline phosphatase; BMD, bone mineral density; FC, free carnitine; IGF-1, insulin-like growth factor-1; iPTH, intact parathyroid hormone; OC, osteocalcin.

The present study showed significant elevation of serum ALP, iPTH and OC levels in CRF patients when compared with the control group and further significant increment of ALP, iPTH and OC levels in HD patients compared with either the control group or the preD patient group. However, a significant decrease in Ca^{2+} level in the same patient groups was reported. These results are in agreement with those of Bayazıt *et al.* [16] who found that serum OC, ALP and iPTH levels were significantly higher in dialysis CRF patients than in the control group. Also, higher than normal bone parameters (OC, ALP, PTH) were registered by Yonova and Dukova [17] in HD patients. These findings suggest that bone metabolism is increased in most HD patients; that is, high bone turnover ROD prevails in HD patients.

Our results are in agreement with the results of several other investigators. Urena *et al.* [18] and Arici *et al.* [19] concluded that serum iPTH and total ALP concentrations were higher in uraemic patients on HD. Rix *et al.* [20] also concluded that iPTH is significantly elevated in preD CRF patients. Regidor *et al.* [21] revealed significant increment of total ALP in HD patients. Kara *et al.* [22] found that in HD patients compared with controls the serum Ca^{2+} levels are lower and serum PTH and ALP levels are higher.

Table 5 Correlations among predialysis patients

	Age	Hb	Urea	Creatinine	BMD	Ca^{2+}	ALP	iPTH	OC	IGF-1
BMD	0.08	0.17	−0.17	−0.22						
Ca^{2+}	0.17	0.21	0.27	−0.26	0.41*					
ALP	−0.03	−0.41	0.02	0.28	−0.45*	−0.91***				
iPTH	−0.02	−0.21	−0.22	0.32	−0.52**	−0.81***	0.75***			
OC	−0.03	0.06	−0.63	−0.34	0.23	−0.15	0.18	0.33		
IGF-1	−0.02	0.49	−0.44	−0.35	0.26	0.04	−0.05	−0.06	0.31	
FC	0.43	0.64	−0.31	−0.55	0.23	0.32	−0.4	−0.17	0.01	0.64**

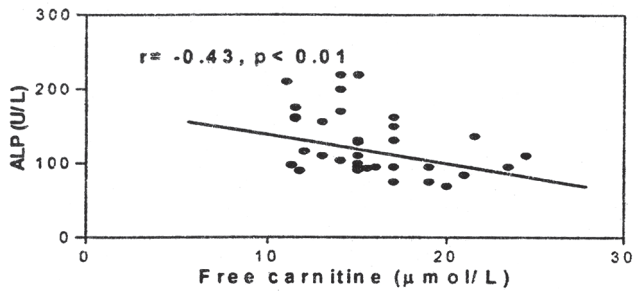
ALP, alkaline phosphatase; BMD, bone mineral density; FC, free carnitine; Hb, haemoglobin; IGF-1, insulin-like growth factor-1; iPTH, intact parathyroid hormone; OC, osteocalcin; *P < 0.05; **P < 0.01; ***P < 0.001.

Table 6 Correlations among haemodialysis patients

	Age	Hb	Urea	Creatinine	BMD	Ca^{2+}	ALP	iPTH	OC	IGF-1
BMD	0.01	0.28	0.2	−0.15						
Ca^{2+}	−0.22	0.01	−0.41	0.21	0.42					
ALP	0.21	−0.17	0.05	−0.19	−0.54*	−0.53*				
iPTH	0.11	−0.12	0.19	−0.22	−0.63**	−0.85***	0.52*			
OC	−0.30	−0.46	0.06	0.22	−0.6**	−0.59**	0.58*	0.73***		
IGF-1	0.10	0.44	−0.16	−0,02	0.49*	0.31	−0.27	−0.32	0.55*	
FC	0.48*	0.67**	−0.18	−0.29	0.09	0.27	−0.04	−0.23	−0.35	0.63**

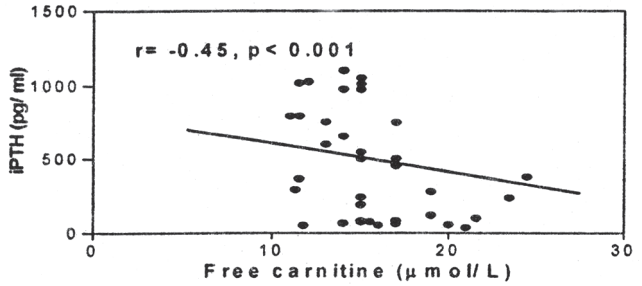
ALP, alkaline phosphatase; BMD, bone mineral density; FC, free carnitine; Hb, haemoglobin; IGF-1, insulin-like growth factor-1; iPTH, intact parathyroid hormone; OC, osteocalcin; *P < 0.05; **P < 0.01.

Figure 1



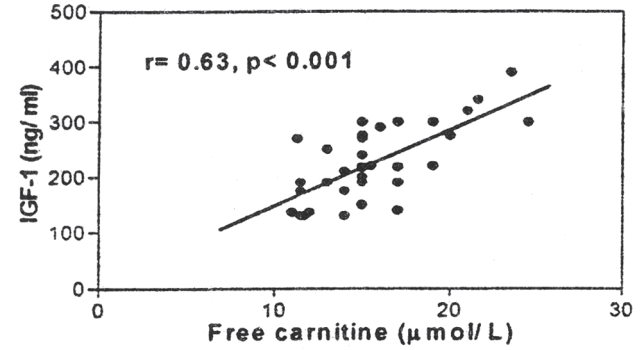
Correlation between free carnitine and alkaline phosphatase (ALP) among chronic renal failure patients.

Figure 2



Correlation between free carnitine and intact parathyroid hormone (iPTH) among chronic renal failure patients.

Figure 3



Correlation between free carnitine and insulin-like growth factor-1 (IGF-1) among chronic renal failure patients.

Recently, Nafie *et al.* [23] reported that there were higher levels of iPTH in HD patients than in preD patients and in preD and HD patients than in controls. There was no statistically significant difference in the level of calcium between HD and preD patients, whereas there were lower levels of calcium in HD patients compared with controls. Also there was no difference in the level of calcium between preD patients and controls.

In this study, serum iPTH had significant positive correlation with ALP and OC in preD and HD CRF patients. Bayazıt *et al.* [16] found significant positive correlation between serum OC and PTH and ALP. Regidor *et al.* [21] found that serum ALP had the strongest correlations with aspartate transaminase (AST) and iPTH. Baskin *et al.* [24] demonstrated that serum OC correlated with bone ALP and iPTH. These authors concluded that bone ALP and OC combined with iPTH level seemed to be useful noninvasive markers of bone metabolism in dialysis patients.

The study by Bakkaloglu *et al.* [25] emphasized the importance of the use of multiple biomarkers in the noninvasive diagnosis of ROD. Indeed, low serum calcium levels and high serum PTH values were the strongest predictors of mineralization defect in patients with bone turnover within the normal range, a finding that should be taken into consideration in view of the availability of therapeutic agents that maintain serum calcium levels within the lower normal range [26,27].

Osteoporosis is a complex multifactorial disease that remains asymptomatic until a fracture occurs, and strategies need to be developed to accurately identify 'high-risk' patients who may benefit from preventive treatments before fractures occur. In osteoporosis, once a fracture occurs, the risk of a subsequent fracture is high. Therefore, the diagnosis of osteoporosis should be made before the first fracture occurs, so that the patient can undertake lifestyle changes and undergo treatment to prevent fractures. The only way to do this is by measuring BMD [28].

Dual-energy X-ray absorptiometry is the standard noninvasive method with which to assess BMD, but it is not always available. Quantitative heel ultrasound is a mobile, relatively inexpensive, easy-to-perform and radiation-free method, which can predict fractures to the same extent as dual-energy X-ray absorptiometry [22]. According to the 2002 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada, it was accepted that 'quantitative heel ultrasound may be considered for diagnosis of osteoporosis' [29].

The results of the present study revealed that the BMD score as measured by calcaneal ultrasonography was significantly lower in HD and preD patients than in control participants and in preD compared with HD patients; further, the HD group was divided into the osteopenia subgroup and the osteoporosis subgroup according to the results of BMD where the osteoporosis subgroup had a significantly lower BMD than the osteopenia subgroup.

These results agreed with those of Kara *et al.* [22]. Depending on the quantitative heel ultrasonography

parameters, mean BMD values of HD patients were significantly lower than those of controls and both osteoporosis and osteopenia were diagnosed in most of their HD patients. Also, Arici *et al.* [19] reported that quantitative heel ultrasonography measurements were markedly reduced in dialysis patients compared with controls. Ha *et al.* [30] confirmed that there was significant bone loss in patients with CRF before the start of dialysis. Arici *et al.* [19] and Kara *et al.* [22] stated that dialysis affects the bone status and HD patients have worse bone mineral metabolism compared with age-matched and sex-matched healthy controls.

Aggarwal *et al*. [28] in their study demonstrated that BMD decreased significantly as the chronic kidney disease (CKD) progressed from stage III to stage V. These findings indicate that alteration in BMD, although beginning early in cases of CKD, is related to the severity of kidney function, and the majority of patients with advanced CKD have reduced bone density and subsequent increased risk for fractures. CKD encompasses several metabolic and hormonal abnormalities, including decreased renal synthesis of 1, 25 (OH)D, hyperphosphatemia, hypocalcemia, increased secretion of PTH, chronic metabolic acidosis, premature hypogonadism and, more recently, recognized 25 (OH) vitamin D deficiency; all may adversely affect the bone remodelling process in one or more of the following ways – increasing bone resorption, decreasing bone formation or impairing mineralization of osteoid. These changes in bone remodelling have the potential to accelerate the deterioration of the bone microstructure that accompanies normal ageing and therefore may prematurely decrease bone quality and strength and increase susceptibility to fragility fracture. They also observed that as severity of CKD increased the total number of patients with reduced bone density (osteopenia and osteoporosis) increased and the prevalence of osteoporosis increased. These findings have important therapeutic implications in terms of defining the population at increased risk for fracture and preventive strategies can be instituted.

Our results showed that the osteoporosis subgroup had significantly increased levels of ALP, iPTH and OC and significant decreases in Ca^{2+} compared with the osteopenia subgroup. Our results also showed that BMD was negatively correlated with ALP, iPTH and OC and positively correlated with Ca^{2+} in preD and HD patients. These results were in agreement with those of Urena *et al.* [18] who found that iPTH and bALP were negatively correlated with BMD in HD patients. Ha *et al*. [30] found inverse linear correlation between total BMD and ALP and iPTH.

Also some previous studies have shown an inverse relationship between BMD and PTH and some other markers. The BMD was negatively correlated with iPTH, which was statistically significant. Elevated PTH levels are catabolic for cortical bone and this accounts for the increased fracture susceptibility noted in patients with CKD [31–33].

In this study, we measure FC and IGF-1 because their deficiency affects bone mass. Our results revealed that in CRF patients FC levels were significantly decreased when compared with the control group and there was a significant decrease in FC in the HD group compared with the preD group and controls. These results are in agreement with those of Wanic-Kossowska *et al.* [34] and Calvani *et al.* [35]. They found that in HD patients serum level of carnitine was significantly lower as compared with the control group of healthy participants and the nondialysed CRF patients.

During the course of human ageing, carnitine concentration in cells diminishes, affecting fatty acid metabolism in various tissues. In particular, bones are adversely affected and continuous reconstructive and metabolic functions of osteoblasts are required for maintenance of bone mass. There is a close correlation between changes in plasma levels of OC and osteoblast activity, and a reduction in OC plasma levels is an indicator of reduced osteoblast activity, which appears to underlie osteoporosis in elderly patients and in postmenopausal women. In a study using experimental animals, administration of l-carnitine was capable of increasing serum OC concentrations, whereas serum OC levels tend to decrease with age in control animals [36]. Moreover, Savica *et al.* [37] have shown that l-carnitine supplementation in HD patients improves BMI and serum albumin.

IGF-1 is one of the most abundant growth factors present in bone that stimulate osteoblast activity, subsequently leading to bone matrix formation and inhibition of bone collagen degradation. IGF-1 is a stimulant factor for renal calcitriol synthesis, enhanced intestinal calcium absorption, bone mineralization and matrix protein synthesis. Several studies have analysed the relationship between IGF-1 levels and bone mass and reported that in different patient groups there is a significant correlation between BMD and IGF-1 levels. There are also studies reporting that even patients with idiopathic osteoporosis have low levels of serum IGF-1 [38].

Results of the present study showed that serum levels of free IGF-1 were significantly decreased in CRF patients when compared with the control group and there was significant positive correlation between BMD and IGF-1 levels in CRF patients on HD.

These results are in agreement with the results of Frystyk *et al.* [39] who observed a reduction of more than 50% in serum levels of free IGF-1 in patients with CRF. Tönshoff *et al.* [40] concluded that growth failure in CRF is mainly due to functional IGF deficiency. Combined therapy with rhGH and rhIGF-1 is therefore a logical approach.

In conclusion, quantitative heel ultrasound is a useful method for evaluating BMD and for detecting regional skeletal changes in CRF patients. Moreover, it can be used to follow bone changes in ROD. The data of the current study suggest that a combination of serum iPTH, ALP and OC levels can predict BMD in CRF patients and may be useful in discriminating patients with normal turnover and defective mineralization from those with normal mineralization. In contrast, a combination of serum iPTH and calcium levels may be used to further identify individuals with poor skeletal mineralization and high fracture risk. Our results also suggested that carnitine treatment is safe and has been shown to be effective in improving certain parameters of interest to dialysis providers, such as erythropoietin resistance, insulin sensitivity, quality of life and hospitalization rate. Also, combined therapy with rhGH and rhIGF-1 is recommended.

Acknowledgements

Conflicts of interest

None declared.

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