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CORRELATION OF FGF23 AND BALP WITH BONE MINERAL DENSITY IN HEMODIALYSIS PATIENTS

KORELACIJA FGF23 I BALP SA MINERALNOM GUSTINOM KOSTIJU KOD PACIJENATA NA HEMODIJALIZI

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Summary

Background: Chronic kidney disease (CKD) is associated with numerous complications such as bone mineral disorder. The aim of our study was to analyze the correlation of bone turnover markers with Bone Mineral Density (BMD) measurements in Tunisian end stage renal diseases (ESRD) patients.
Methods: This study included 100 ESRD Tunisian patients. Their estimated glomerular filtration rate (eGFR) was < 15 mL $\times$ min^{-1} $\times$ (1.73 m^2)$^{-1}$, which requires hemodialysis. Bone-specific alkaline phosphatase (BALP) serum concentration was determined with a chemiluminescence immunoassay. Fibroblast Growth Factor 23 (FGF23) serum was assessed by Enzyme-Linked Immunosorbent Assay method. The serum levels of 25-Hydroxyvitamin D (25(OH)D), intact parathyroid hormone (iPTH) and Beta cross-laps (CTX) was measured by Electrochemiluminescence Technology. DEXA (dual-energy x-ray absorptiometry) technique was used to evaluate BMD.
Results: We observed a statistically significant negative correlation between BALP levels and total body BMD (r =

Kratak sadržaj

Uvod: Hronično bubrežno oboljenje (CKD) je praćeno brojnim komplikacijama kao što je poremećaj minerala kostiju. Svha ovog izučavanja je bila da analizira korelaciju markera promena u kostima sa mineralnom gustinom kostiju (BMD) i stupnja renalnih oboljenja kod pacijenata u Tunisu.
Metode: Proučavanje je uključilo 100 ESRD tuniskih pacijenata. Njihova prosečna brzina glomerularne filtracije (eGFR) bila je < 15 mL $\times$ min^{-1} $\times$ (1,73 m^2)$^{-1}$ što je zahtevalo hemodijalizu. Koštana alkalna fosfataza (BLAP) određena je u serumu hemilumiiscentnom imuno metodom. Faktor rasta fibroblasta 23 (FGF23) u serumu određen je enzimskom imunosorbetnom metodom. Nivoi 25-hidroksivitamina D (25(OH)D), intaktnog paratiroidnog hormona (iPTH) i beta-kros-lapsa (CTX) izmereni su elektrohemiluminiscentnom DEXA tehnologijom (dual-energy x-ray apsorpciometrijom) kako bi se procenila BMD.
Rezultati: Nađena je značajno negativna statistička korelacija BALP nivoa i ukupnog telesnog BMD (r= -0,268: P = 0,015) u femoralnom delu vrata (FN) (r = -0,219; P = 0,037). BALP je bila u negativnoj korelaciji sa ukupnom BMD

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-0.268; P = 0.015) particularly in femoral neck (FN) (r = -0.219; P = 0.037). BALP concentrations were negatively associated with total BMD especially in FN for patients with BMI < 30, FGF23 concentrations were also negatively correlated with BMD in lumbar spine site (LS) (r = -0.209; P = 0.046). For osteopenic patients we found an inverse correlation between 25(OH)D concentrations and BMD in LS position (r = -0.336; P = 0.038). In men group, we have also found a negative correlation between iPTH and total BMD (r = -0.326; P = 0.015). However we found a positive correlation between calcium expression and BMD in LS site (r = 0.270; P = 0.031).
Conclusions: FGF23 and BALP can predict bone loss in ESRD through their strong correlation with BMD in LS and FN sites respectively.

Keywords: 25-Hydroxyvitamin D, bone alkaline phosphatase, bone mineral density, bone mineral disorder, chronic kidney disease, fibroblast growth factor 23

naročito kod FN pacijenata sa BMI < 30, FGF23 koncentracije su bile takođe u negativnoj korelaciji sa BMD u lumbalnom delu (LS) (r = -0,209, P = 0,046). Kod osteopeničnih pacijenata nađene je inverzna korelacija između 25(OH)D koncentracije i DMD u LS položaju (r = -0,336; P = 0,038). U grupi muških osoba nađena je negativna korelacija između iPTH i ukupnog BMD (r = -0,326; P = 0,015). Međutim, nađena je pozitivna korelacija između ekspresije kalcijuma i BMD u LS (r = 0,270: P = 0,031).
Zaključak: FGF23 i BALP mogu da predvide gubitak kosti kod ESRD preko izrazite korelacije sa BMD u LS i FN položajima.

Ključne reči: 25-hidroksivitamin D, koštana alkalna fosfataza, miniralna gustina kosti, hronično oboljenje bubrega, faktor rasta fibroblasta 23

Introduction

New biochemical markers are being developed as non invasive exploration methods of bone turnover particularly in renal diseases (1). Chronic kidney diseases (CKD) is a complex renal pathology associated with numerous complications such as kidney failure and bone mineral disorder (CKD-MBD) (2). The alteration of kidney function is related to a progressive bone loss, leading to the onset of osteoporosis (3). The abnormalities in bone volume were positively correlated with CKD severity, especially during end stage renal disease (ESRD) (4–6). An overexpression of bone turnover markers (BTM) was also observed during CKD. These markers indicating bone remodeling include calcium; phosphate; iPTH, alkaline phosphatase (PAL) and BALP (7–9).

In CKD PAL and BALP measurements were widely recommended, to evaluate not only in the bone mineral status disorder, but also in the rate of vascular calcification (10). BALP is an enzyme that promotes bone mineralization by inactivating pyrophosphate and osteopontin, which are both inhibitors of bone mineralization (11, 12). I's role is essential in determining the rate of bone formation and turnover (13). While BALP is a marker of bone formation, serum CTX, a collagen-degradation product, is a marker of bone resorption (14, 15).

FGF23, formed by osteocyte and osteoblast cells, is implicated in addition to BALP in bone remodeling (13, 16, 17). In fact, FGF23 has been considered as a bone marker and has a crucial role, not only in mineral balance, but also in bone mineralization and vascular calcification (18, 19).

It's also a regulator of vitamin D metabolism through a different bone-kidney axis. It inhibits renal tubular reabsorption of phosphate, impacting both calcium and phosphate bone transportation, and induces reduction of 1,25(OH)2D concentrations (20–23).

As a result, the rise in iPTH can be attributed not only to the absence of the direct impact of 1,25(OH)D on parathyroid gland (PTG) and bone, but also to the intestinal absorption of calcium (24). Moreover, the decrease of serum 1-25-hydroxy-vitamin D, commonly observed in CKD, may have an important role in the pathogenesis of osteoporosis in such patients (25).

Those markers can probably provide a better control of BMD to hemodialysis patients. In addition, the use of DEXA is recommended by multiple guidelines, thanks to its efficiency in discriminating bone loss in ESRD patients (8).

The aim of this study was to evaluate the correlation of bone turnover markers with DEXA measurements in Tunisian ESRD patients.

Materials and Methods

Patients

This study included 100 hemodialysis patients. A male predominance was found (64%) with a sex-ratio of 1.77. The mean age was 57.5 ± 15.00 years old; with extremes going from 23 to 94 years old and the mean disease duration was 7.20 ± 4.20 years. All patients were in ESRD. Before starting hemodialysis, their eGFR value was less than < 15 mL $\times$ min^{-1} $\times$ $(1.73$ m$^2)^{-1}$. Our population was classified into two major groups: diabetics (N = 60) and non-diabetics (N = 40).

Patients have not received vitamin D supplementation in the last six months. Their clinical and demographic data were gathered in *Table I*. They were followed-up at the Nephrology Department of the RABTA Hospital and they were undergoing hemodialysis three times a week during 4 to 5 hours.

Exclusion criteria were patients with liver disease, cirrhosis, gastrectomy, parathyroidectomy, hysterectomy and evolutive neoplasias as well as those under glucocorticoides. Patients' demographic and medical information including age, sex, height, weight, smoking history, heart disease, hypertension, diabetes, hyperlipidemia and drug history were gathered and recorded in predefined data sheets.

This study was approved by the local ethics committee of the RABTA hospital, and a written informed consent was obtained from all the participants conformably to the ethical standards and the Declaration of Helsinki Principles.

Human samples

All blood samples were obtained after 8 hours an overnight fasting, just before starting hemodialysis. Subsequent plasma and serum were separated within 30 minutes and the samples were conserved frozen at -80 °C until assays were realized.

Serum markers

Serum BALP was determined by chemiluminescence immunoassay (Access-Ostase® immunoassay, Beckman-Coulter Inc., USA.) on the ACCESS immunoassay system; ACCESS® Ostase is a chemiluminescent immunoassay with paramagnetic particles for the quantitative determination of serum BALP using the ACCESS Analyzer. The test is a one-step enzyme immunoassay. Whereas serum FGF23 was assessed by ELISA method (Demeditec®, Germany). It's a Sandwich ELISA method, which measures the amount of antigen between two layers of antibodies. The antigen to be measured contain two antigenic sites capable of binding to antibody.

The following parameters were measured by Integra 400 Plus analyzer (Germany, Roche Diagnostics®): Total serum calcium (endpoint method with 5-nitro-5'-methyl-BAPTA (NM-BAPTA), Creatinine (kinetic compensated Jaffe method); Phosphate (endpoint method with ammonium molybdate) and PAL (kinetic method with p-nitrophenylphosphate).

Serum 25(OH)D, iPTH and CTX were measured by Electrochemiluminescence Technology: (Cobas e411 analyzer, Germany, Roche Diagnostics®). This method is an immunological test for a quantitative determination of 25(OH)D, iPTH and CTX base on a competition principle for 25(OH)D and a »sandwich« for the two others assays. The inter-assay coefficient of variation for 25(OH)D, iPTH, CTX, BALP and FGF23 were lower than 11%, 4%, 9%, 6.5% and 12% respectively.

Measurements for BMD

Dual-Energy X-ray Absorptiometry technique was used for the evaluation of BMD and the detection of osteoporosis. DEXA measurements in lumbar spine and femoral neck were assessed using WHO (World Health Organization) criteria as a cutoff point. BMD results were evaluated according to the T-score based on the number of standard deviations (SDs). The normal BMD is defined by a T-scores higher than -1, A T-score lower than -1 defines osteopenia, while osteoporosis is associated with values lower then -2,5 (26).

According to the WHO traditional densitometric classification, for DEXA measurements, when the studied population includes post-menopausal women or men aged ≥50 years old; the T-score values show the type of bone loss. This diagnosis of WHO should also be applied to menopausal women (26). In postmenopausal women, the diagnosis of bone loss is made normal when the values of T-scores will be equal or exceed -1,0 SD. In men over the age of 50, osteopenia (low bone density) is defined by T-scores values between -1,0 and- 2,5 SDs. In menopausal transition osteoporosis is determined by values of T-scores equal or lower than -2,5 SDs (27).

Statistical analysis

Means and SD were determined for the quantitative variables. Data was analyzed using SPSS software version 24. The comparative study was realized using the Student's t-test for quantitative variables and the X^2 test or the Fischer's exact test for qualitative variables. The correlation between parameters was evaluated by Pearson correlation analysis. The significance level P was set at 0.05.

Results

The means of BMD in the lumbar spine, femoral neck and in the total body were respectively 1.14 ± 0.22 g/cm^2; 0.92 ± 0.16 g/cm^2 and 1.11 ± 0.12 g/cm^2, The means of LS, FN, T and Z score were respectively -0.58 ± 1.81 g/cm^2; 0.12 ± 1.74 g/cm^2; -1.08 ± 1.26 g/cm^2 and -0.38 ± 1.15 g/cm^2 (Table I).

For the lumbar spine T score, 58 patients (58%) had normal bone profile, 29 (29%) patients were osteopenic and 13 (13%) were osteoporotic. As for the femoral neck T-score, 51 (51%) had normal bone profile, 36 (36%) patients were osteopenic and 13 (13%) were osteoporotic (Table I). Twenty-two osteoporotic patients where identified (68% female versus 32% male). Their mean age was 68.23 ± 10.69 years old; higher than observed in normal profile patients (52.70 ± 16.26 years) and in osteopenic ones (56.34 ± 12.82 years). Moreover, the duration of hemodialysis expressed in months was

Table I Clinical and biological characteristics of hemodialysis patients.

Patients Characteristic	Mean ± SD (N %)
Population numbers	N = 100
Biological parameters	
Age (years)	57.50 ± 15.01
Gender (Female /Male)	36/64 (36% / 64%)
Tabacco	48 (48%)
Alcohol	19 (19%)
Duration of dialysis (months)	79.91 ± 51.13
Weight (m^2)	70.27 ± 12.45
Height (kg)	1.64 ± 0.10
BMI (kg/m^2)	26.30 ± 4.95
Clinical parameters	
CTX (ng/mL)	1.99 ± 1.40
iPTH (pmol/L)	32.50 ± 33.42
25(OH)D (nmol/L)	37.37 ± 25.45
BALP (ng/mL)	24.31 ± 19.04
FGF23 (pg/mL)	212.31 ± 228.90
Calcium (mmol/L)	2.21 ± 0.20
Phosphate (mmol/L)	1.96 ± 1.06
Creatinine (μmol/L)	765.02 ± 606.52
PAL (UI/L)	100.90 ± 48.33
Bone mineral density data [range]	
LS-BMD (g/cm^2)	1.14 ± 0.22 [0.63–1.95]
LS-T	-0.58 ± 1.8 [1-4.6–6.1]
LS-Z	0.12 ± 1.74 [-3.8–7.2]
FN-BMD (g/cm^2)	0.92 ± 0.16 [0.64–1.4]
FN-T	-1.08 ± 1.26 [-3.2–2.4]
FN-Z	-0.38 ± 1.15 [-2.2–3.3]
TOTAL-BMD (g/cm^2)	1.11 ± 0.12 [0.86–1.41]
LS-T LS-T ≥-1	58 (58%)
-2.5 < LS-T < -1	29 (29%)
LS-T -2.5	13 (13%)
LS-T ≥-1	58 (58%)
FN-T LS-T ≥-1	51 (51%)
-2.5 < LS-T < -1	36 (36%)
LS-T -2.5	13 (13%)

BMI: Body Mass index; CTX: Beta cross laps; iPTH: parathyroid hormone; 25(OH)D:25-hydroxyvitamin D; FGF23: fibroblast growth factor 23; PAL: alkaline phosphatase; BALP: bone alkaline phosphatase, BMD: Bone Mineral Density; LS-BMD: Bone Mineral Density in Lumbar spine site; FN-BMD: Bone Mineral Density in Femoral neck; LS-T: T score in Lumbar spine site, FN-T: T score in Femoral neck; LS-Z: Z score in Lumbar spine site, FN-Z: Z score in Femoral neck

longer in osteoporotic patients (102.45 ± 60.42) compared to osteopenic patients (66.21 ± 31.94) (*Table II*).

BMD in LS and FN sites were significantly lower in women. Significant differences between male and female patients in BMD T score and Z score were observed (P < 0.05) both in LS and FN sites. The mean values of bone markers (iPTH, CTX, BALP and FGF23) were higher in female patients but not statistically significant (P > 0.05) (*Table III*).

There was a significant negative correlation between BALP concentrations and total body bone mineral density (r = -0.268; P = 0.015) particularly in the femoral neck (r = -0.219; P = 0.037). Similarly, a significant negative correlation between FGF23 concentrations and the BMD in lumbar spine [(r = -0.209; P = 0,046 and T scores (r = -0.213; P = 0.041)] was retained (*Figure I*).

Additionally, low 25(OH)D concentrations were negatively correlated with LS-Z in osteopenic patients (r = -0.336; P = 0.038) (*Table IV*). Moreover, in osteoporotic patients, we found that phosphate concentrations were negatively associated with FN-T (r = -0.569; P = 0.005); FN-BMD (r = -0,575; P = 0.005) and total BMD (r = -0.603; P = 0.010).

Stratification based on different biological and clinical parameters revealed many different correlations. We observed a negative correlation between calcium concentrations and total BMD in women group (r = -0.346; P = 0.048). In men group, here was a negative correlation between iPTH and total BMD (r = -0.326; P = 0.015). However, a positive correlation was detected between both calcium and BMD in lumbar spine site (r = 0.270; P = 0.031) and total BMD (r = 0.295; P = 0.027) (*Table IV*).

In patients younger than 55 years old, we found that serum concentration of BALP is negatively correlated with FN-T (r = -0.386; P = 0.018) and total BMD in FN (r = -0.349; P = 0.034). In addition, a significant negative association between creatinine concentrations and Z-LS was detected (r = -0.457; P = 0.0027). In patients older than 55 years, a negative correlation was found between iPTH and total BMD (r = -0.322; P = 0.020) (*Table V*).

Analysis based on BMI factor indicated that in patients with BMI lower than 30, there were positive correlations between 25(OH)D and FN-T, FN-Z and FN-BMD [(r = 0.367; P = 0.015); (r = 0.354; P = 0.019); (r = 0.355; P = 0.019)] respectively. In patients with BMI < 30, BALP was negatively associated with FN-T (r = -0.319; P = 0.042), FN-Z (r = -0.337; P = 0.031), FN-BMD (r = -0.311; P = 0.048) and Total BMD (r = -0.371; P = 0,023). In this group, the iPTH concentrations were negatively correlated with total BMD (r = -0.375; P = 0.018), while a positive significant association was found

Table II Distribution of clinical characteristics of patients according to BMD.

	Normal Mean ± SD / N (%)	Osteopenia Mean ± SD / N (%)	Osteoporosis Mean ± SD / N (%)
Sex (M/F)	30/10 (75% / 25%)	27/11 (71% / 29%)	7 / 15 (32% / 68%)
Age (years)	52.70 ± 16.26	56.34 ± 12.82	68.23 ± 10.69
Tabacco	22 (55%)	20 (53%)	6 (27%)
Alcohol	9 (23%)	10 (26%)	0
Physical activity	5 (13%)	5 (13%)	2 (9%)
Duration of Dialysis (months)	80.53 ± 56.86	66.21 ± 31.94	102.45 ± 60.42
Weight (kg)	71.19 ± 10.57	69.32 ± 12.89	70.23 ± 15.09
Height (m^2)	1.68 ± 0.09	1.64 ± 0.09	1.57 ± 0.09
BMI (kg/m^2)	25.40 ± 3.78	25.81 ± 4.41	28.79 ± 6.81
CTX (ng/mL)	2.02 ± 1.44	2.05 ± 1.44	1.81 ± 1.29
iPTH (pmol/L)	32.23 ± 36.41	34.38 ± 30.40	29.61 ± 34.23
25(OH)D (nmol/L)	36.17 ± 23.77	37.42 ± 25.55	39.50 ± 29.12
BALP (ng/mL)	22.47 ± 18.89	21.84 ± 11.95	31.91 ± 26.72
FGF23 (pg/mL)	171.38 ± 186.52	214.95 ± 238.08	300.60 ± 281.06
Calcium (mmol/L)	2.21 ± 0.16	2.21 ± 0.17	2.25 ± 0.29
Phosphate (mmol/L)	1.94 ± 1.09	1.99 ± 1.08	1.95 ± 1.02
Creatinine (µmol/L)	728.253 ± 269.006	696.870 ± 311.260	949.610 ± 1175.74
PAL (UI/L)	93.44 ± 48.26	113.76 ± 45.58	91.50 ± 53.59

BMI: Body Mass index; CTX: Beta cross laps; iPTH: parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D; FGF23: fibroblast growth factor 23; PAL: alkaline phosphatase; BALP: bone alkaline phosphatase

Table III Comparison of clinical and biological characteristics between male and female.

	Female (N = 36) Mean ± SD	Male (N = 64) Mean ± SD	P
Age (years)	60.08 ± 16.36	56.05 ± 14.12	0.198
Duration of dialysis (months)	75.25 ± 52.26	82.53 ± 50.71	0.497
Weight (kg)	66.39 ± 14.73	72.45 ± 10.47	0.034
Height (m^2)	1.55 ± 0.07	1.69 ± 0.08	< 0.001
BMI (kg/m^2)	27.65 ± 6.59	25.55 ± 3.58	0.083
CTX (ng/mL)	2.08 ± 1.48	1.93 ± 1.36	0.617
iPTH (pmol/L)	30.97 ± 29.53	33.35 ± 35.59	0.736
25(OH)D (nmol/L)	34.90 ± 26.02	38.77 ± 25.22	0.468
BALP (ng/mL)	28.65 ± 21.86	22.28 ± 17.38	0.138
FGF23 (pg/mL)	248.05 ± 254.36	193.24 ± 213.91	0.276
Calcium (mmol/L)	2.23 ± 0.24	2.20 ± 0.16	0.440
Phosphate (mmol/L)	1.88 ± 0.99	2.01 ± 1.10	0.571
PAL (UI/L)	99.03 ± 50.74	102.07 ± 47.69	0.842
Creatinine (µmol/L)	861.20 ± 937.411	710.92 ± 285.710	0.236
LS-BMD (g/cm^2)	1.02 ± 0.22	1.20 ± 0.19	< 0.001
LS-T	-1.32 ± 1.85	-0.16 ± 1.65	0.002
LS-Z	-0.14 ± 1.48	0.27 ± 1.86	0.251
FN-BMD (g/cm^2)	0.85 ± 0.16	0.95 ± 0.16	0.002
FN-T	-1.25 ± 1.32	-0.98 ± 1.22	0.291
FN-Z	-0.51 ± 1.03	-0.30 ± 1.22	0.387
Total-BMD (g/cm^2)	1.04 ± 0.10	1.15 ± 0.11	< 0.001

BMI: Body Mass index; CTX: Beta cross laps; iPTH: parathyroid hormone; 25(OH)D:25-hydroxyvitamin D; FGF23: fibroblast growth factor 23; PAL: alkaline phosphatase; BALP: bone alkaline phosphatase, BMD: Bone Mineral Density; LS-BMD: Bone Mineral Density in Lumbar spine site; FN-BMD: Bone Mineral Density in Femoral neck; LS-T: T score in Lumbar spine site, FN-T: T score in Femoral neck; LS-Z: Z score in Lumbar spine site, FN-Z: Z score in Femoral neck; P: signification

Table IV Correlation between biochemical parameters and the BMD according to gender.

		Women							Male						
		LS-Z	LS-T	FN-T	FN-Z	LS-BMD	FN-BMD	Total-BMD	LS-Z	LS-T	FN-T	FN-Z	LS-BMD	FN-BMD	Total-BMD
25(OH)D	r	0.057	0.105	0.225	0.191	0.098	0.216	0.172	-0.184	-0.161	0.003	0.001	-0.122	0.029	-0.085
	P	0.741	0.542	0.187	0.266	0.567	0.205	0.339	0.146	0.203	0.982	0.993	0.337	0.818	0.533
Phosphate	r	0.189	0.086	-0.087	-0.029	0.077	-0.096	-0.164	-0.109	-0.135	-0.074	-0.107	-0.164	-0.088	-0.140
	P	0.270	0.619	0.614	0.866	0.655	0.577	0.361	0.391	0.288	0.561	0.401	0.194	0.488	0.304
BALP	r	0.021	-0.085	-0.153	-0.046	-0.091	-0.154	-0.237	-0.069	-0.137	-0.177	-0.153	-0.123	-0.197	-0.221
	P	0.915	0.660	0.429	0.813	0.637	0.425	0.225	0.595	0.287	0.168	0.234	0.341	0.125	0.108
iPTH	r	-0.123	-0.084	-0.010	-0.028	-0.086	-0.014	-0.238	-0.075	-0.083	-0.161	-0.183	-0.101	-0.165	-0.326
	P	0.483	0.632	0.957	0.872	0.622	0.934	0.190	0.558	0.519	0.209	0.151	0.429	0.196	0.015*
PAL	r	-0.011	0.012	0.162	0.132	0.010	0.167	0.350	-0.209	-0.219	-0.151	-0.166	-0,219	-0.168	-0.177
	P	0.966	0.965	0.533	0.613	0.970	0.522	0.183	0.297	0.272	0.452	0.408	0.272	0.400	0.418
FGF23	r	-0.217	-0.203	-0.093	-0.100	-0.198	-0.091	-0.169	-0.172	-0.176	-0.167	-0.164	-0.166	-0.152	-0.137
	P	0.233	0.265	0.614	0.584	0.277	0.621	0.371	0.189	0.178	0.201	0.210	0.203	0.244	0.327
CTX	r	0.114	0.165	0.164	0.136	0.159	0.154	-0.041	-0.008	-0.104	-0.126	-0,082	-0.122	-0.139	-0.257
	P	0.507	0.336	0.341	0.430	0.354	0.368	0.819	0.952	0.417	0.327	0.523	0,342	0,278	0.057
Creatinine	r	-0.088	-0.130	0.009	0.062	-0.124	0.012	-0.161	-0.149	-0.053	0.102	-0.004	-0.037	0.094	0.050
	P	0.609	0.450	0.960	0.718	0.472	0.945	0.369	0.241	0.678	0.421	0.974	0.770	0.459	0,715
Calcium	r	0.094	-0.077	-0.274	-0.119	-0.077	-0.264	-0.346	0.115	0.209	0.223	0.137	0.270	0.231	0.295
	P	0.587	0.653	0.106	0.488	0.653	0.120	0.048*	0.365	0.098	0.076	0.282	0.031*	0.066	0.027*

CTX: Beta cross laps; iPTH: parathyroid hormone; 25(OH)D:25-hydroxyvitamin D; FGF23: fibroblast growth factor 23; PAL: alkaline phosphatase; BALP: bone alkaline phosphatase, BMD: Bone Mineral Density; LS-BMD: Bone Mineral Density in Lumbar spine site; FN-BMD: Bone Mineral Density in Femoral neck; LS-T: T score in Lumbar spine site, FN-T: T score in Femoral neck; LS-Z: Z score in Lumbar spine site, FN-Z: Z score in Femoral neck; p: signification

* P value < 0.05; r: Correlation coefficient

Table V Correlation between biochemical parameters and BMD according to patients age.

		Age < 55 years							Age > 55 years						
		LS-Z	LS-T	FN-T	FN-Z	LS-BMD	FN-BMD	TOTAL BMD	LS-Z	LS-T	FN-T	FN-Z	LS-BMD	FN-BMD	TOTAL BMD
25(OH)D	r	-0.046	-0.088	0.109	0.140	-0.086	0.105	0.001	-0.128	-0.005	0.049	-0.001	0.041	0.092	0.085
	P	0.777	0.586	0.497	0.381	0.594	0.513	0.998	0.333	0.968	0.713	0.995	0.757	0.489	0.550
Phosphate	r	-0.125	-0.122	-0.019	-0.056	-0.126	-0.032	-0.186	0.074	0.002	-0.229	-0.134	-0.013	-0.203	-0.107
	P	0.435	0.448	0.904	0.727	0.432	0.841	0.270	0.577	0.990	0.081	0.310	0.923	0.124	0.449
BALP	r	0.010	-0.106	-0.386	-0.250	-0.104	-0.349	-0.310	-0.107	-0.223	-0.056	-0.038	-0.223	-0.158	-0.253
	P	0.950	0.531	0.018*	0.136	0.540	0.034*	0.078	0.441	0.105	0.687	0.787	0.104	0.253	0.079
iPTH	r	-0.139	-0.053	-0.149	-0.214	-0.032	-0.110	-0.206	-0.039	-0.092	-0.118	-0.082	-0.117	-0.143	-0.322
	P	0.385	0.741	0.352	0.178	0.844	0.492	0.220	0.771	0.495	0.380	0.542	0.386	0.290	0.022*
PAL	r	-0.203	-0.197	-0.114	-0.070	-0.163	-0.031	-0.058	-0.067	-0.022	0.058	-0.046	-0.039	-0.009	0.181
	P	0.434	0.448	0.663	0.788	0.531	0.907	0.838	0.740	0.912	0.772	0.821	0.846	0.964	0.396
FGF23	r	-0.202	-0.212	-0.073	-0.092	-0.222	-0.101	-0.219	-0.188	-0.225	-0.259	-0.213	-0.210	-0.247	-0.204
	P	0.224	0.202	0.661	0.583	0.180	0.545	0.206	0.172	0.102	0.058	0.122	0.127	0.071	0.163
CTX	r	-0.053	-0.015	-0.074	-0.116	-0.005	-0.069	-0.201	0.111	-0.034	-0.073	0.061	-0.063	-0.125	-0.245
	P	0.742	0.925	0.646	0.468	0.977	0.668	0.233	0.405	0.801	0.586	0.649	0.636	0.350	0.082
Creatinine	r	-0.457	-0.312	-0.064	-0.186	-0.309	-0.071	-0.130	-0.022	-0.085	0.061	0.086	-0.085	0.026	-0.146
	P	0.002*	0.047*	0.690	0.243	0.049	0.657	0.444	0.867	0.523	0.648	0.515	0.520	0.843	0.303
Calcium	r	0.183	0.024	-0.053	-0.184	0.016	-0.180	-0.128	0.016	0.038	0.088	0.076	0.088	0.088	0.029
	P	0.228	0.874	0.729	0.226	0.918	0.236	0.427	0.902	0.777	0.508	0.567	0.508	0.505	0.838

BMI: Body Mass index; CTX: Beta cross laps; iPTH: parathyroid hormone; 25(OH)D:25-hydroxyvitamin D; FGF23: fibroblast growth factor 23; PAL: alkaline phosphatase; BALP: bone alkaline phosphatase , BMD: Bone Mineral Density; LS-BMD: Bone Mineral Density in Lumbar spine site; FN-BMD: Bone Mineral Density in Femoral neck; LS-T: T score in Lumbar spine site, FN-T: T score in Femoral neck; LS-Z: Z score in Lumbar spine site, FN-Z: Z score in Femoral neck; p: signification
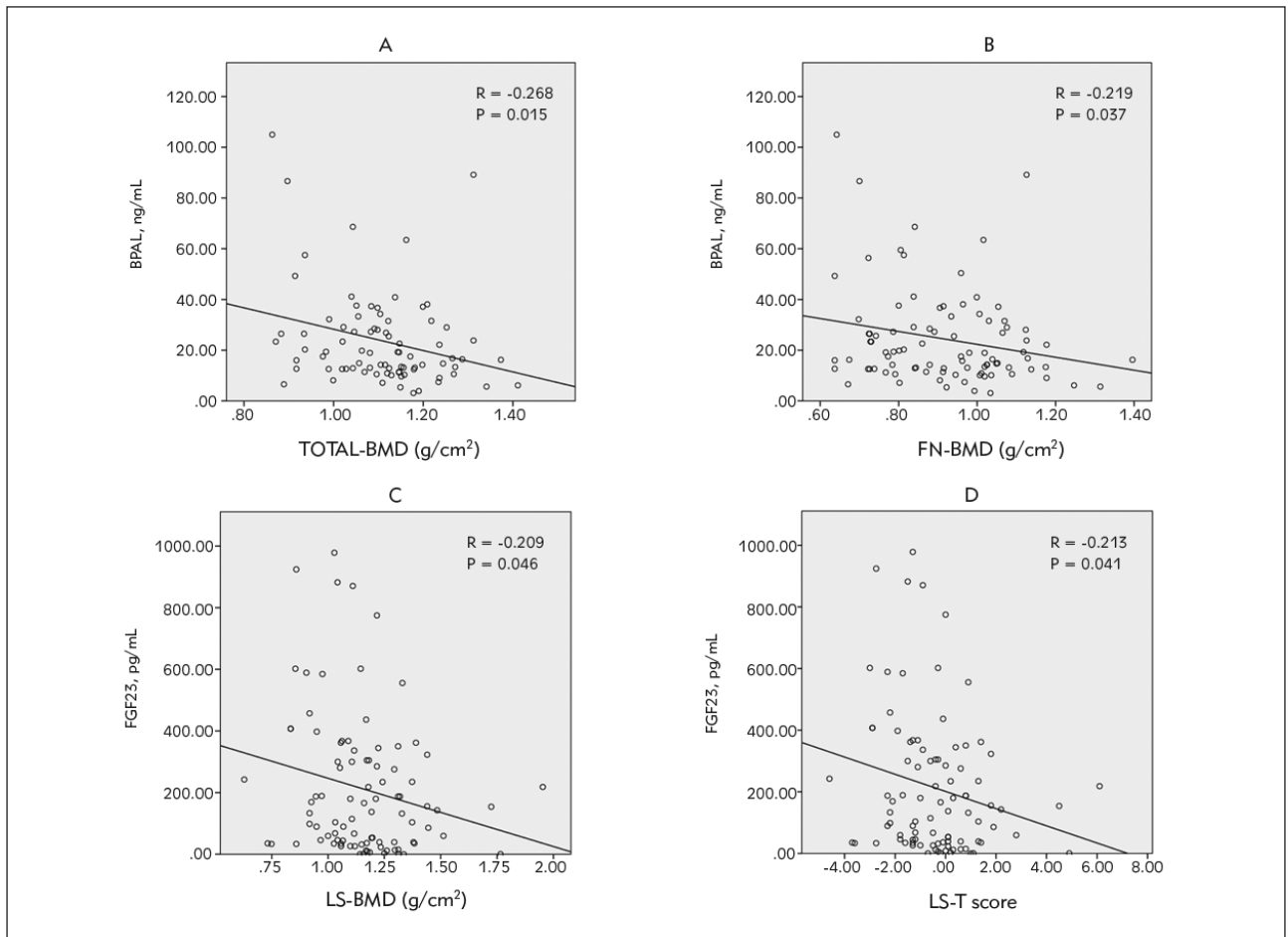
* P value < 0,05; r: Correlation coefficient

Figure 1 Correlation between BALP, FGF23 levels and BMD in hemodialysis patients
A: Correlation between BALP levels and total BMD in hemodialysis patients; B: Correlation between BALP and BMD in FN in hemodialysis patients; C: Correlation between FGF23 levels and LS-BMD in hemodialysis patients; D: Correlation between FGF23 levels and LS-T scores in hemodialysis patients

between creatinine and FN-BMD (r = 0.305; P = 0.046). Besides we detected a positive correlation between calcium concentrations and FN-T (r = 0.302; P = 0.049). In obese patients with a BMI higher than 30, a negative correlation was found between 25(OH)D and LS-Z (r = -0.278; P = 0.036).

Discussion

In our study, we found that serum BALP and FGF23 concentrations were associated with a loss of BMD in femur and spine regions, respectively. Actually, in our research total body BMD measurements were negatively correlated with elevated BALP concentrations in Tunisian population. This was confirmed in many previous studies (9, 10, 28). Furthermore, we observed a significant negative correlation between an increased BALP concentration and the BMD only in the femoral neck site. Our findings were similar to those reported by Fidan et al. (7) in Turkey. However, many studies revealed that BALP was a predictive factor of bone loss, mainly in cortical sites,

and was weakly associated with BMD essentially in trabecular sites (9, 29). These contradictory results could be explained by the difference between of studied populations, methods of analysis of bone loss measurements and the wide choice of measurement sites established by researchers. This negative correlation is affected by gender and age factors; Particularly old patients. But, lower BMI has no impact on this correlation when compared to overweight patient groups. Otherwise, serum BALP is the most sensitive and specific bone marker in the detection of bone turnover disorder in Tunisian patients with CKD and probably for patient with low BMI and younger than 55 years old. Besides, no correlation was established between PAL and total BMD neither in our results nor in others' (30, 31). Nevertheless, other studies showed that PAL concentrations were inversely correlated to the total BMD (32, 33).

We also investigated the expression of FGF23 in Tunisian ESRD patients, as a bone marker that has a major role in bone turnover, and it is enclosed in bone mineral status (20, 21). We found that the increased

concentrations of FGF23 were correlated with BMD loss in the lumbar spine site. However, this correlation is affected by age, sex and BMI. This result was partly confirmed by Malluche et al. (10) who correlated the rise of FGF23 concentration with BMD loss in spine not only at baseline but also after 12 months of follow-up.

In addition, and according to several studies, the low 25(OH)D concentration was one of the factors leading to osteoporosis not only in healthy persons but also in patients with CKD. This marker can be used for a primary detection of this bone pathology (25, 34). The decrease in bone mineralization and the reduction in calcium absorption is also one of the causes of this pathology.

In ESRD Tunisian patients, osteopenia status is related to low bone mass and reduced 25(OH)D levels. Thus, a vitamin D supplementation is needed. Alfacalcidol, Calcitriol, Paricalcitol and other vitamin D analogues should be recommended to prevent the risk of osteoporosis (35). Furthermore, we found a negative correlation between iPTH concentrations and bone loss in various BMD measurement sites, in particular for men or old and overweight ESRD patients. However, our results were different from those reported in other studies (3, 7, 36).

Our findings revealed the absence of significant correlation between the elevation of CTX concentrations and BMD in both the femur and the spine regions. In fact, the higher expression of CTX levels is caused by the decline of renal activity (37). Thus, no relationship between CTX and BMD measurements in hemodialysis was reported though their major implication in vascular calcification, and on secondary hyperparathyroidism (38, 39).

In conclusion, FGF23 and BALP can predict bone loss in end stages of CKD through their strong correlation with the lumbar spine and femoral neck sites respectively.

Human and Animal Rights: The study was approved by the local ethics committee of the RABTA hospital, Tunisia.

Informed Consent: a written informed consent was obtained from all the participants conformably to the ethical standards and the Declaration of Helsinki Principles.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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