

Body mass index is inversely associated with osteoblastic activity in patients undergoing hemodialysis

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Abstract. Triggered by the association of malnutrition with the incidence of fractures in patients undergoing hemodialysis (HD), the present study evaluated whether the body mass index (BMI) is associated with serum markers of osteoblastic or osteoclastic activity in this population. The levels of the osteoblastic markers, total procollagen type-1 aminoterminal propeptide (P1NP) and osteocalcin (OC), the osteoclastic marker, β -isomerized C-terminal cross-linked peptide of collagen type I (β -CTX), and those of the intact parathyroid hormone (iPTH) were measured in the serum of 59 patients undergoing HD. iPTH, P1NP, OC and β -CTX were intercorrelated. BMI inversely correlated with the osteoblastic markers, P1NP and OC, but not with the osteoclastic marker, β -CTX. Age negatively correlated with OC, β -CTX and iPTH. The levels of P1NP, OC and β -CTX were lower in patients with diabetes mellitus. BMI affected the osteoblastic markers independently of age, diabetes mellitus and iPTH. Thus, BMI inversely correlates with markers of osteoblastic activity in patients undergoing HD. However, other studies are required to confirm causality in this correlation. If confirmed, then enhancing osteoblastic activity by improving the nutritional status may become another therapeutic strategy against chronic kidney disease-mineral bone disease.

Introduction

Bone disease is relatively common among patients undergoing hemodialysis (HD) who suffer an increased frequency of fractures (1). In routine clinical practice, intact parathyroid hormone (iPTH) is the primary marker that dictates the therapeutic maneuvers in chronic kidney disease (CKD)-mineral bone disease (MBD). However, clinical studies have

demonstrated that the specificity and sensitivity of iPTH to detect the nature of CKD-MBD are modest (2,3). In addition, targeting iPTH levels mainly affects osteoclastic activity, since PTH exerts its action on the bone mainly by increasing the expression and, to a certain extent, the secretion of receptor activator of nuclear factor- κ B ligand (RANKL) by osteoblasts. In turn, RANKL binds to RANK in osteoclasts, enhancing their function (4).

Of note, in the general population and in patients undergoing HD (5-8), anthropometric indicators of nutritional status are positively associated with increased bone loss, but are negatively associated with fractures. In addition, decreased protein intake is associated with reduced bone density. The latter has been attributed to the reduced maturation and activity of osteoblasts (9-11). Since the protein-energy wasting syndrome is prevalent among patients undergoing HD (12), the present study evaluated whether body mass index (BMI), a readily available nutritional indicator, correlates with serum markers of bone osteoblastic and osteoclastic activity in patients undergoing HD.

In order to assess osteoblastic activity, the levels of total procollagen type-1 aminoterminal propeptide (P1NP), a product derived by the cleavage of procollagen produced by osteoblasts (13), were measured. In addition, the levels of osteocalcin (OC), another protein produced by osteoblasts were measured. OC is the second most abundant bone protein, next to collagen, and contributes to bone mineralization (14). To assess osteoclastic activity, the levels of β -isomerized C-terminal cross-linked peptide of collagen type I (β -CTX), fragments of collagen produced during its degradation by osteoclasts, were measured (15). A recent study demonstrated that the accuracy of the aforementioned markers in detecting the nature of CKD-MBD is comparable to that of iPTH (3).

Patients and methods

Patients. A total of 59 patients undergoing HD (mean age, 60.5 \pm 12.5 years; 40 males) participated in the present study. The cause of end-stage renal disease was diabetes mellitus (DM) in 21 patients, primary glomerulonephritis in 9 patients, hypertension in 7 patients, interstitial nephritis in 5 patients, obstructive nephropathy in 3 patients, autosomal dominant polycystic kidney disease in 4 patients, and was unknown

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in 10 patients. All patients were >18 years of age, and none of them suffered from any malignancy or active infectious disease.

The patients underwent 4-h HD sessions, three times a week, for at least 1 year prior to the study. Polysulfone low-flux dialyzers and a bicarbonate dialysate containing 1.5 mmol/l calcium were used. The urea reduction ratio at the time of the study was $65.4 \pm 7.4\%$. Serum calcium levels were 9.60 ± 0.64 mg/dl, serum phosphorous levels were 5.92 ± 1.78 mg/dl and serum albumin levels were 3.99 ± 0.29 g/dl. All patients had anuria. None of the patients suffered from any active infection, malignancy or autoimmune disease, and none had a history of parathyroidectomy. BMI was calculated using the following equation: $BMI = \text{body weight (Kg)} : \text{height (m)}^2$.

None of the patients received corticosteroids, cytotoxic drugs, warfarin, anticonvulsants, antidepressants, hormone replacement therapy, bisphosphonates, or calcimimetics for at least 6 months prior to the study. Sevelamer hydrochloride (Renagel; Genzyme Europe) or lanthanum carbonate (Fosrenol; Shire Pharmaceuticals Ltd.) were used as phosphate binders. A written informed consent was obtained from each individual, and the Ethics Committee of the University of Thessaly, Faculty of Medicine approved the study protocol (approval no. 558/10-2-2017).

Blood sample analyses. Blood samples were obtained at the onset of the second dialysis session of the week, and serum was stored at -80°C . Immunoassays for measuring P1NP, N-terminal midfragment OC, β -CTx, iPTH and 25-hydroxy-vitamin D (25(OH)D) were performed using an ELECSYS 2010 automatic analyzer (Roche Diagnostics GmbH).

Statistical analysis. IBM SPSS Statistics for Windows software, version 26 (IBM Corp.) was used for the statistical analysis. The one-sample Kolmogorov-Smirnov test was used to evaluate whether the variables were normally distributed. Apart from 25(OH)D, the variables did not follow a normal distribution. Thus, non-parametric tests were used for further statistical analyses. Spearman's Rho correlation coefficient was calculated for detecting correlations, and the Mann-Whitney U test was used for comparing values. Linear regression with robust standard error estimation analysis was used to evaluate the independent effect of BMI on the osteoblastic or osteoclastic markers (16). The results are expressed as the median (interquartile range), and a two-sided $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The median, ranges and the interquartile range values of the evaluated factors are depicted in Table I. The correlations between BMI and the evaluated biochemical markers of bone metabolism are presented in Table II.

BMI was not associated with the age of the patients. In addition, BMI did not differ markedly among the patients undergoing HD with or without DM [24.62 (8.25) vs. 23.89 (4.74) in patients with or without DM, respectively, $P = 0.868$] (Fig. 1).

However, age correlated with OC ($Rho = -0.523$, $P < 0.001$), β -CTx ($Rho = -0.475$, $P < 0.001$) and iPTH ($Rho = -0.306$, $P = 0.019$)

Table I. Median, range and interquartile range values of the evaluated variables.

Variable	Median	Range	Interquartile range
Age (years)	65	49	18
BMI	24.57	28.40	7.40
P1NP (ng/ml)	267.60	857.29	208.20
OC (ng/ml)	221.00	755.58	210.40
β -CTx (ng/ml)	1.89	4.47	1.35
iPTH (pg/ml)	181.00	1083.05	198.47
25(OH)D (ng/ml)	21.64	19.66	17.41

25(OH)D, 25-hydroxy vitamin D; BMI, body mass index; iPTH, intact parathyroid hormone; OC, osteocalcin; P1NP, procollagen type-1 aminoterminal propeptide; β -CTx, β -isomerized C-terminal cross-linked peptide of collagen type I.

(Table II). From the evaluated biochemical markers, diabetes mellitus affected OC, P1NP and β -CTx. OC levels were lower in patients with DM [106.0 (77.2) vs. 286.9 (173) ng/ml; $P < 0.001$], P1NP levels were lower in patients with DM [154.6 (73.1) vs. 372.9 (250.9) ng/ml; $P < 0.01$] and β -CTx levels were also lower in patients with DM [1.23 (1.22) vs. 1.97 (1.68) ng/ml; $P < 0.001$], whereas the iPTH levels did not differ significantly between patients with or without DM [180.1 (126.9) vs. 195.1 (237.2) pg/ml; $P = 0.359$] (Fig. 1).

Contrary to patients' age, OC, P1NP and β -CTx levels did not differ according to the patients' sex. OC levels were 228.4 (105.3) ng/ml in males and 219.2 (134.9) ng/ml in females ($P = 0.903$). P1NP levels were 257.2 (167.7) ng/ml in males and 285.2 (226.6) ng/ml in females ($P = 0.604$). Finally, β -CTx levels were 1.85 (1.27) ng/ml in males and 1.89 (1.23) ng/ml in females ($P = 0.916$) (Table III). As regards 25(OH)D, its serum concentration was 21.64 (17.41) ng/ml and it did not correlate with OC ($Rho = -0.172$, $P = 0.194$), P1NP ($Rho = -0.229$, $P = 0.08$), or β -CTx ($Rho = -0.035$, $P = 0.793$) (Table II).

Furthermore, linear regression with robust standard error estimation analysis was applied to evaluate whether the nutritional indicator, BMI, affects the osteoblastic markers, P1NP and OC, independently of age and DM. The usual marker of CKD-MBD and regulator of bone metabolism, iPTH, was also included in the model, since it correlated with P1NP and OC. Regression analysis revealed that BMI affected the P1NP and OC levels independently of age, DM or iPTH (Table IV).

Discussion

Triggered by studies demonstrating an association between nutritional status and the incidence of fractures in patients undergoing HD (6-8), the present study evaluated the correlation between nutritional status and serum markers of osteoblastic or osteoclastic activity in patients undergoing HD. The present study used the readily available nutritional marker, BMI, widely used in the general population and patients undergoing HD.

To evaluate osteoblastic activity, the P1NP and OC levels were assessed (13,14), while for osteoclastic activity, β -CTx

Table II. Correlations among the evaluated variables.

Variable		Age, years	BMI	P1NP	OC	β -CTx	iPTH
Age, years	Rho						
	P-value						
BMI	Rho	0.076					
	P-value	0.567					
P1NP	Rho	-0.226	-.430				
	P-value	0.085	<0.001				
OC	Rho	-0.523	-0.356	0.769			
	P-value	<0.001	0.006	<0.001			
β -CTx	Rho	-0.475	-0.245	0.543	0.731		
	P-value	<0.001	0.061	<0.001	<0.001		
iPTH	Rho	-0.306	-0.053	0.338	0.525	0.620	
	P-value	0.019	0.693	0.009	<0.001	<0.001	
25(OH)D	Rho	-0.058	0.062	-0.229	-0.172	-0.035	0.076
	P-value	0.665	0.642	0.08	0.194	0.793	0.566

Values in bold font indicate statistically significant differences ($P < 0.05$). 25(OH)D, 25-hydroxy vitamin D; BMI, body mass index; iPTH, intact parathyroid hormone; OC, osteocalcin; P1NP, procollagen type-1 aminoterminal propeptide; β -CTx, β -isomerized C-terminal cross-linked peptide of collagen type I.

Table III. Median (interquartile range) values of OC, P1NP and β -CTx in male and female patients.

Variable	Males	Females	P-value
OC (ng/ml)	228.4 (105.3)	219.2 (134.9)	0.903
P1NP (ng/ml)	257.2 (167.7)	285.2 (226.6)	0.604
β -CTx (ng/ml)	1.85 (1.27)	1.89 (1.23)	0.916

OC, osteocalcin; P1NP, procollagen type-1 aminoterminal propeptide; β -CTx, β -isomerized C-terminal cross-linked peptide of collagen type I.

levels were assessed (15). These factors have a similar predictive value with iPTH, the primary marker used in clinical practice to classify the nature of CKD-MBD (3). To strengthen the reliability of the results, patients undergoing HD who had anuria were enrolled, since P1NP and β -CTx are subjected to renal clearance (3).

BMI inversely correlated with the osteoblastic markers, P1NP and OC, whereas no correlation was detected with the osteoclastic marker, β -CTx. Thus, a non-optimal nutritional status in patients undergoing HD may contribute to CKD-MBD by decreasing osteoblastic activity. Experimental and clinical studies in other populations detected such a correlation and incriminated the diminishing insulin-like growth factor-1 (IGF-1) level due to a low protein intake (9-11). In patients undergoing HD, a low IGF-1 level is associated with malnutrition, reduced bone mineral density and mortality (17). Protein-energy wasting syndrome is prevalent in patients undergoing HD (12), and malnutrition is a cause of adynamic bone disorder (18), which is characterized by a reduced number

Table IV. Univariate linear regression analysis with robust standard errors estimation analysis on the effect of BMI, age, iPTH and DM on P1NP or OC.

A, Dependent variable: P1NP

Parameter	B	Robust S.E.	t value	P-value
Intercept	917.401	145.004	6.327	<0.001
BMI	-17.332	3.267	-5.305	<0.001
Age	-2.574	1.678	-1.534	0.131
DM	-206.667	38.429	-5.378	<0.001
iPTH	0.421	0.205	2.057	0.045

R-squared=0.563 (adjusted R-squared=0.530) $P < 0.001$.

B, Dependent variable: OC

Parameter	B	Robust S.E.	t value	P-value
Intercept	729.272	102.787	7.095	<0.001
BMI	-9.739	1.257	-7.745	<0.001
Age	-4.680	1.161	-4.032	<0.001
DM	-155.544	18.510	-8.403	<0.001
iPTH	0.491	0.053	9.186	<0.001

R-squared=0.802 (adjusted R-squared=0.787) $P < 0.001$.

Values in bold font indicate statistically significant differences ($P < 0.05$). BMI, body mass index; iPTH, intact parathyroid hormone; OC, osteocalcin; P1NP, procollagen type-1 aminoterminal propeptide.

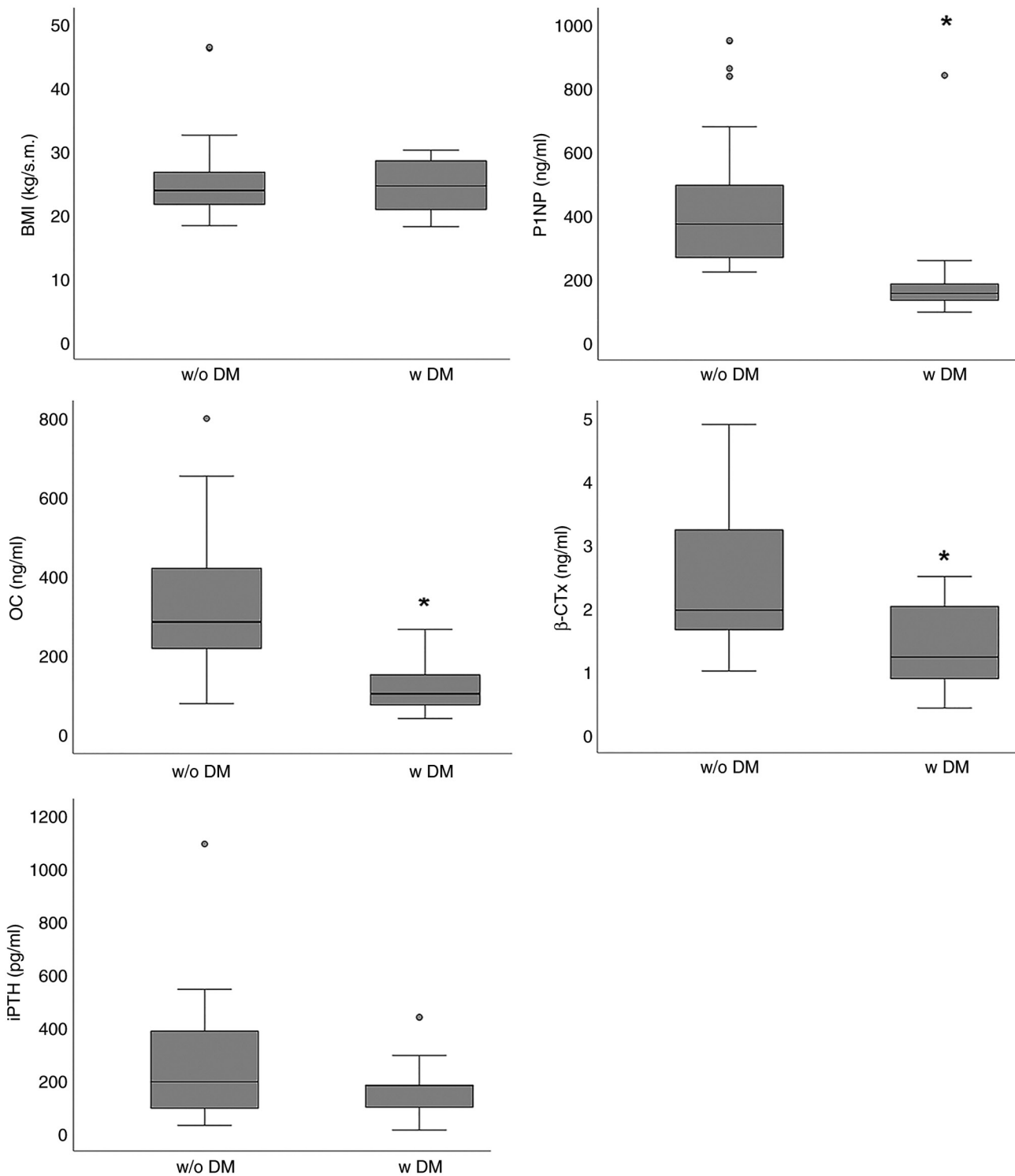


Figure 1. Whisker plots demonstrating the effects of DM on the evaluated factors. The levels of P1NP, OC and β -CTx were lower in patients with DM. BMI and the levels of iPTH did not markedly differ in patients with or without DM. * $P < 0.05$. DM, diabetes mellitus; P1NP, procollagen type-1 aminoterminal propeptide; OC, osteocalcin; β -CTx, β -isomerized C-terminal cross-linked peptide of collagen type I; BMI, body mass index; iPTH, intact parathyroid hormone.

of osteoblasts (19). Further more extensive studies are required however, to confirm the causality between the nutritional status and osteoblastic activity. This would lead to the development of novel therapeutic strategies for CKD-MBD. Contrary to current practice, which targets iPTH and osteoclastic activity, these interventions will target osteoblastic activity.

In the present study, an intercorrelation between iPTH and all the aforementioned osteoblastic and osteoclastic

markers was detected. These correlations are likely the result of the coupled osteoclastic and osteoblastic activity. In the bone-forming units, osteoblasts assemble only when iPTH-driven osteoclasts have completed resorption. The result is a new packet of bone that replaces the removed older bone (19).

In the present study, the serum 25(OH)D level was 21.84 ± 5.24 ng/ml. Thus, 25(OH)D was below the recommended

lower limit of 30 ng/ml in the majority of patients. A high incidence of 25(OH)D insufficiency is common among patients undergoing HD (20). Notably, in the present study 25(OH)D was not correlated with OC, P1NP or β -CTx. Previous studies have also demonstrated a lack of an association between serum 25(OH)D levels and markers of osteoblastic or osteoclastic activity (21,22).

In the present study, BMI was not associated with the patients' age. In addition, the BMI values did not differ among the patients undergoing HD with or without DM. In addition, BMI was not correlated with iPTH. However, age and DM affect bone metabolism in patients undergoing HD, and adynamic bone disease is more common among elderly and diabetic patients undergoing HD (23). Indeed, in the present study, age negatively correlated with OC, β -CTx and iPTH in the patients undergoing HD. Furthermore, the patients with DM undergoing HD had lower median OC, P1NP and β -CTx values. Of note, in the present cohort of patients undergoing HD, and contrary to patients' age, the OC, P1NP and β -CTx levels did not differ according to the patients' sex. The latter may result from heavily disrupted bone metabolism that characterizes patients undergoing HD, which may prevail to hormonal sex differences.

Regression analysis revealed that BMI determines P1NP and OC levels significantly and independently of age, DM and the levels of iPTH. As it is already known, CKD-MBD significantly contributes to mortality in patients undergoing HD (24). Hence, to a certain extent, the effect of nutritional status on bone metabolism detected in the present study may explain the reverse epidemiology of the higher the BMI, the lower the mortality observed in this population (25).

Certainly, the findings obtained herein require validation from further studies using larger cohorts of patients undergoing HD. Additionally, the simultaneous assessments of bone structure alongside measurements of bone mineral density or bone biopsy with histological examinations, which were not conducted in the present study, would provide greater clarity on this matter. In conclusion, BMI inversely correlates with markers of osteoblastic activity in patients undergoing HD. Further studies are warranted to confirm causality in this correlation. This could lead to the development of strategies with which to enhance osteoblastic activity by improving nutritional status in patients with CKD-MBD.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

TE designed the study. TE, GA, GP, EN and IS contributed to the acquisition, analysis and interpretation of the data. TE

wrote the manuscript. TE and GA confirm the authenticity of all the row data. All authors drafted the manuscript, critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted according to the Declaration of Helsinki, and was approved by the Ethics Committee of the University of Thessaly, Faculty of Medicine (approval no. 558/10-2-2017). A written informed consent was obtained from all subjects involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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