Plasma Serotonin and Markers of Bone Formation and Bone Resorption in Hemodialysis Patients

Theodoros Eleftheriadis,[1,2] Georgia Antoniadi,[1]
Vassilios Liakopoulos,[1] Theodora Sparopoulou,[2]
Ioannis Stefanidis,[1] Grammati Galaktidou[2]

[1]Department of Nephrology, Medical School, University of Thessaly, Larissa, Greece
[2]Research Institute, Theagenion Anticancer Hospital, Thessaloniki, Greece

Keywords. hemodialysis, bone metabolism, serotonin

Original Paper

Introduction. Serotonin receptors are present in osteoblasts and osteoclasts, and serotonin affects bone metabolism. The association of plasma serotonin with markers of bone formation and bone resorption in hemodialysis patients was evaluated.
Materials and Methods. Twenty-four hemodialysis patients (11 diabetics) and 22 healthy volunteers were enrolled into the study. Serotonin was assessed in platelet-free plasma, whereas the markers of osteoblastic activity N-terminal midfragment osteocalcin and total procollagen type-1 aminoterminal propeptide as well as the marker of osteoclastic activity β-isomerized C-terminal cross-linked peptide of collagen type I were measured in serum. Serum intact parathyroid hormone was also assessed.
Results. Serotonin did not significantly differ between hemodialysis patients and healthy volunteers. All evaluated markers of bone metabolism and intact parathyroid hormone were much higher in hemodialysis patients. Serotonin was significantly correlated with all evaluated markers of bone metabolism in hemodialysis patients. Serotonin was reversely related to the patients' age. Serotonin, osteocalcin, procollagen type-1 aminoterminal propeptide, and β-isomerized C-terminal cross-linked peptide of collagen type I were much lower in diabetic hemodialysis patients.
Conclusions. Serotonin may increase both bone formation and bone resorption in hemodialysis patients. The reverse relation of serotonin to patients' age as well as its lower levels in diabetic hemodialysis patients indicate that low plasma serotonin may contribute to the higher incidence of low-turnover bone disease that characterizes old and diabetic hemodialysis patients.

IJKD 2013;7:36-41
www.ijkd.org

INTRODUCTION

Renal osteodystrophy is the term used to describe the various skeletal abnormalities that are seen in association with chronic kidney disease (CKD). Histological findings classify renal osteodystrophy in 2 major types. High-turnover bone disease, which is characterized by abnormal and increased bone remodeling, includes osteitis fibrosa and mixed disorders. Low-turnover bone disease, which is characterized by decreased bone mineralization and formation, includes osteomalacia and nowadays, at the post-aluminum era, mainly adynamic bone disorder.[1]

Parathyroid hormone synthesis and secretion as well as bone sensitivity to this hormone are deregulated in CKD. Because this hormone has a predominant effect on bone turnover, intact parathyroid hormone (PTH) is the primary clinical

marker used in the assessment of bone turnover in hemodialysis patients.[2] However, bone turnover cannot be predicted by serum PTH measurements in 30% of hemodialysis patients.[3] Thus the research continues.

Although serotonin obtained its claim to fame as a neurotransmitter, it is produced mainly (95%) in the periphery by enterochromaffin cells in the gastrointestinal tract. Because serotonin does not cross the blood-brain barrier, centrally and peripherally synthesized serotonin acts in isolation. A proportion of serotonin produced in the periphery is taken up by the platelets leaving only a small percentage (< 5%) free in plasma.[4] Several studies support a significant role for the last serotonin fraction in bone metabolism.[5-8]

Serotonin receptors have been identified in osteoblasts, osteoclasts, and osteocytes.[9-11] Some experimental studies support that serotonin enhance bone formation,[12,13] whereas others support the opposite.[14] Similarly, an effect of serotonin on bone resorption pathways has been described.[11,13] Studies in patients treated with selective serotonin reuptake inhibitors, which increase plasma serotonin levels, showed that use of selective serotonin reuptake inhibitors, but not tricyclic antidepressants, was associated with increased rates of bone loss at the hip,[15] reduced bone mineral density at multiple skeletal sites in men,[16] and a 2-fold increase in the risk of clinical fragility fractures.[17] Generally, the risk of hip and femur fractures are higher in patients treated with selective serotonin reuptake inhibitors than in patients treated with tricyclic antidepressants.[18,19] In a population-based sample of 275 women, serum serotonin was a negative predictor of femur neck total and trabecular volumetric bone mineral density, as well as of trabecular thickness at the radius.[20]

The aim of the present study was to evaluate the possible impact of peripheral serotonin on bone turnover in hemodialysis patients. To our knowledge, this has never been evaluated before in this population. For this purpose, serotonin in platelet-free plasma was assessed. The N-terminal midfragment (NMID) osteocalcin was used as a marker of bone formation, since osteocalcin is a noncollagenous protein that is present in bone matrix and is produced by osteoblasts.[21] Total procollagen type 1 aminoterminal propeptide (P1NP) was also used as a marker of bone formation, since type 1 procollagen is synthesized by osteoblasts and P1NP derived during the conversion of procollagen to collagen and its subsequent incorporation into the bone matrix.[22] Beta-isomerized C-terminal cross-linked peptide of collagen type I (β-CTx) was used as a marker of bone resorption, since it is a collagen derivative that is released during bone resorption by the osteoclasts.[23] The above markers of bone metabolism, as well as intact PTH, have been correlated well with bone histomorphometric and histodynamic parameters in hemodialysis patients.[24-27]

MATERIALS AND METHODS

Patients

Twenty-four hemodialysis patients (mean age, 62.5 ± 9.5 years, 8 women) and 22 healthy volunteers (mean age, 58.9 ± 5.9 years, 9 women) participated in the study. Healthy volunteers were chosen from the personnel of the hospital, according to their medical records, physical examination, and recent routine laboratory tests. An informed consent was obtained from each individual enrolled into the study and the hospital ethics committee gave its approval to the study protocol.

All patients were selected to be anuric, since NMID osteocalcin, P1NP, and β-CTx are secreted in the urine and differences in kidney function influence their serum levels.[28] Patients underwent regular hemodialysis with polysulfone low-flux dialysis membranes (F low-flux series, Fresenius Medical Care, Bad Homburg, Germany) and with a bicarbonate dialysis solution containing 2.5 mEq/L or 3 mEq/L calcium for 4-hour sessions, 3 times a week, and for at least 1 year prior to the study. The KT/V according to the second-generation natural logarithmic formula based on the single pool urea kinetic model was 1.22 ± 0.23. At the time of blood samples collection, serum calcium level was 9.38 ± 0.48 mg/dL, serum phosphate was 6.09 ± 1.99 mg/dL, and serum albumin was 3.95 ± 0.30 g/dL. None of the patients suffered from infection, malignancy, or autoimmune disease, and none had a history of parathyroidectomy. None of the patients were receiving corticosteroids, cytotoxic drugs, warfarin, anticonvulsants, antidepressants, hormone replacement therapy, or biphosphonates for at least 6 months prior to the study. Patients treated with vitamin D receptor activators or calcimimetics were excluded as well. Sevelamer hydrochloride (Renagel, Genzyme Pharmaceuticals,

Naarden Netherland) or lanthanum carbonate (Fosrenol, Shire Pharmaceuticals Group, Chineham, Basingstoke, UK) were used as phosphate binders.

Methods

Blood samples were drawn in the morning just before the onset of the second dialysis session of the week. A 10-mL sample of blood was drawn from each individual. Serum was obtained from 6 mL of blood sample, and plasma was obtained from the remaining 4 mL of blood. Platelet-free plasma and serum were stored at -80°C.

Immunoassays for measuring serum intact PTH, NMID osteocalcin, P1NP, and β-CTx were performed in an ELECSYS 2010 automatic analyser (Roche Diagnostics GmbH, Mannheim, Germany). Serotonin was assessed in platelet-free plasma by means of enzyme-linked immunosorbent assay using a commercially available kit (Serotonin ELISA, IBL, Hamburg, Germany).

Statistical Analyses

The normal distribution of the variables in the healthy volunteers and hemodialysis patients group was evaluated and confirmed using the 1-sample Kolmogorov-Smirnov test. In both groups, all the evaluated variables were normally distributed. For comparison of mean values between hemodialysis patients and healthy volunteers, the unpaired *t* test (2-sided, including Levene test for equality of variances) was used. However, because of the small number of the subjects in the case of comparison of mean values between diabetic and nondiabetic hemodialysis patients, the nonparametric Mann-Whitney U test was used for comparisons. Results were expressed as mean ± standard deviation and differences were considered significant when 2-sided *P* value was less than .05. Correlations between variables in the hemodialysis patients group were assessed using the Pearson correlation coefficient test. Multiple linear regression analysis was applied in order to evaluate the specific impact of serotonin on bone turnover in hemodialysis patients. Statistical analysis was carried out with the SPSS software (Statistical Package for the Social Sciences, version 16.5, SPSS Inc, Chicago, Ill, USA).

RESULTS

Participants

Twenty-four hemodialysis patients and 22 health volunteers participated in the study. The two groups did not differ significantly regarding age ($P = .14$). The cause of end-stage renal disease was diabetes mellitus in 11 patients, primary glomerulonephritis in 4 patients, interstitial nephritis in 2 patients, hypertension in 2 patients, obstructive nephropathy in 1 patient, and unknown in 4 patients. Diabetic and nondiabetic patients did not differ regarding age (mean age, 65.3 ± 7.2 years versus 60.1 ± 10.8 years, respectively, $P = .19$).

Comparisons Between Hemodialysis Patients and Healthy Individuals

Plasma serotonin levels were higher in hemodialysis patients, but the difference did not reach statistical significance (8.50 ± 5.12 ng/mL versus 6.44 ± 1.54 ng/mL, $P = .07$). Intact PTH and the bone turnover markers levels were all increased in hemodialysis patients. Osteocalcin level was much higher in hemodialysis patients (298.28 ± 128.17 ng/mL versus 17.56 ± 6.44 ng/mL, $P < .001$). The level of P1NP was also increased in hemodialysis patients (326.22 ± 232.38 ng/mL versus 37.26 ± 10.17 ng/mL, $P < .001$). The same was detected for β-CTx (1.99 ± 1.02 ng/mL versus 0.26 ± 0.10 ng/mL, $P < .001$). As it was expected, intact PTH level was increased in hemodialysis patients (209.52 ± 143.85 pg/mL versus 45.36 ± 19.93 pg/mL, $P < .001$; Table 1).

Relationships Between Parameters Among Hemodialysis Patients

There were significant relationships between osteocalcin and P1NP or β-CTx (r = 0.632, $P = .001$ and r = 0.819, $P < 0.001$; respectively) and between P1NP and β-CTx (r = 0.629, $P = .001$). Osteocalcin correlated with intact PTH (r = 0.608, $P = .002$) and with serotonin (r = 0.514, $P = .01$). Additionally, P1NP correlated with intact PTH (r = 0.399, $P = .05$)

Table 1. Plasma Serotonin and Serum Markers of Bone Turnover in Hemodialysis Patients and Healthy Volunteers*

Parameter	Hemodialysis Patients	Healthy Volunteers	*P*
Serotonin, ng/mL	8.50 ± 5.12	6.44 ± 1.54	.07
Osteocalcin, ng/mL	298.28 ± 128.17	17.56 ± 6.44	< .001
P1NP, ng/mL	326.22 ± 232.38	37.26 ± 10.17	< .001
β-CTx, ng/mL	1.99 ± 1.02	0.26 ± 0.10	< .001
intact PTH, pg/mL	209.52 ± 143.85	45.36 ± 19.93	< .001

*P1NP indicates procollagen type 1 aminoterminal propeptide; β-CTx, β-isomerized C-terminal cross-linked peptide of collagen type I; and PTH, parathyroid hormone.

Table 2. Correlations Between Serum Markers of Bone Turnover and Serum Intact Parathyroid Hormone and Plasma Serotonin in Hemodialysis Patients*

Parameter	Intact PTH		Serotonin	
	r	*P*	r	*P*
Osteocalcin	0.608	.002	0.514	.01
P1NP	0.399	.05	0.521	.009
β-CTx	0.541	.006	0.621	.001

*PTH indicates parathyroid hormone; P1NP, procollagen type 1 aminoterminal propeptide; and β-CTx, β-isomerized C-terminal cross-linked peptide of collagen type I.

and with serotonin (r = 0.521, *P* = .009). Finally, β-CTx significantly correlated with intact PTH (r = 0.541, *P* = .006) and with serotonin as well (r = 0.621, *P* = .001; Table 2).

No significant relationship was detected between serotonin and intact PTH (r = 0.303, *P* = .15). Serotonin was reversely related to patients' age (r = -0.474, *P* = .02), which was the case for β-CTx as well (r = -0.429, *P* = .04), whereas intact PTH and P1NP were not linked to patients' age (r = -0.279, *P* = .19 and r = -0.039, *P* = .86; respectively). Interestingly, osteocalcin almost reached statistical significance regarding its reverse correlation with patients' age (r = -0.379, *P* = .07).

Combined Effect of Serotonin and Parathyroid Hormone on Bone Turnover in Hemodialysis Patients

Multiple linear regression analysis was applied in order to define the impact of plasma serotonin on bone turnover in hemodialysis patients. The markers of osteoblastic activity osteocalcin and P1NP and the marker of osteoclastic activity β-CTx were used as dependent variables, whereas plasma serotonin and serum intact PTH were the independent variables. In all three cases, serotonin had a strong impact independent of intact PTH on bone turnover markers. The standardized beta coefficient was 0.363 (*P* = .04) and the adjusted R^2 was 0.441 for osteocalcin. The standardized beta was 0.440 (*P* = .03) and the adjusted R^2 was 0.272 for P1NP. The standardized beta was 0.503 (*P* = .004) and the adjusted R^2 was 0.478 for β-CTx.

Comparisons Between Diabetic and Nondiabetic Hemodialysis Patients

Compared to nondiabetic hemodialysis patients, serotonin was lower in diabetic hemodialysis patients (10.25 ± 6.30 ng/mL versus 6.42 ± 1.99 ng/mL, *P* = .04). Interestingly, compared to healthy volunteers serotonin was higher in nondiabetic hemodialysis patients (8.50 ± 5.12 ng/mL versus 10.25 ± 6.30 ng/mL, *P* = .009). On the contrary, serotonin did not differ significantly between healthy volunteers and diabetic hemodialysis patients (8.50 ± 5.12 ng/mL versus 6.42 ± 1.99 ng/mL, *P* = 0.72).

Serum levels of all the evaluated bone turnover markers were significantly lower in diabetic hemodialysis patients. Osteocalcin level was 282.16 ± 124.03 ng/mL in nondiabetics and 120.96 ± 61.98 ng/mL in diabetics (*P* < .001). The P1NP level was 412.75 ± 221.87 ng/mL in nondiabetics and 223.96 ± 209.50 ng/mL in diabetics (*P* = .001). Finally, β-CTx level was 2.45 ± 1.07 ng/mL in nondiabetics and 1.44 ± 0.63 ng/mL in diabetics (*P* = .009). On the contrary, no significant difference was detected in intact PTH levels between diabetics and nondiabetics (189.34 ± 114.68 pg/mL versus 226.60 ± 167.36 pg/mL, respectively; *P* = .78; Table 3).

DISCUSSION

In the present study, the possible effect of circulating serotonin on bone metabolism in hemodialysis patients, who are characterized by certain skeletal abnormalities,[1] was evaluated. To our knowledge, this has never been evaluated before in this population. The results were intriguing since plasma serotonin was found to be associated with both markers of bone formation and bone resorption in these patients.

Compared to healthy volunteers, NMID osteocalcin, P1NP and β-CTx were significantly increased in hemodialysis patients. This finding per se does not mean that bone turnover is higher in all hemodialysis patients, because the above markers

Table 3. Plasma Serotonin and Serum Markers of Bone Turnover in Diabetic and Nondiabetic Hemodialysis Patients*

Parameter	Nondiabetic Patients	Diabetic Patients	*P*
Serotonin, ng/mL	10.25 ± 6.30	6.42 ± 1.99	.04
osteocalcin, ng/mL	282.16 ± 124.03	120.96 ± 61.98	< .001
P1NP, ng/mL	412.75 ± 221.87	223.96 ± 209.50	.001
β-CTx, ng/mL	2.45 ± 1.07	1.44 ± 0.63	.009
intact PTH, pg/mL	226.60 ± 167.36	189.34 ± 114.68	.78

*P1NP indicates procollagen type 1 aminoterminal propeptide; β-CTx, β-isomerized C-terminal cross-linked peptide of collagen type I; and PTH, parathyroid hormone

are secreted in the urine and decreased kidney function leads to their accumulation in serum.[28] However, the validity of the above markers to assess bone metabolism in hemodialysis patients has been confirmed in previous studies,[24-27] and this is also supported by the strong relationship between these markers and intact PTH detected in the present study.

The relationship between osteocalcin and P1NP was expected, since both are markers of osteoblastic activity. The relationship between the above two markers and β-CTx, which is a marker of osteoclastic activity, is also rational, since osteoblastic and osteoclastic activities are coupled. In the bone forming units, osteoblasts assemble only when osteoclasts have completed resorption. The end result is a new packet of bone that has replaced the older bone that was removed.[29]

Regarding platelet-free plasma serotonin, the results were intriguing. Compared to healthy volunteers, serotonin levels were higher in hemodialysis patients but the difference did not reach statistical significance. Considering that the exact mechanism for the trend for increased plasma serotonin in hemodialysis patients is not known, and only hemodialysis patients enrolled into the study, conclusions cannot be extrapolated for the differential role of CKD or of hemodialysis procedure per se in the above observation.

In hemodialysis patients, serotonin was associated with all evaluated markers of bone metabolism, indicating that possibly this substance increases both osteoblastic and osteoclastic activity in this population. Multivariable regression analysis revealed that this effect is significant and independent of the effect of intact PTH, which is a well-known potent stimulator of bone turnover in this population.[1,2] No relation between serotonin and intact PTH was detected and consequently peripheral serotonin may play a role in cases where bone turnover cannot be predicted by serum PTH measurements. These cases account to about one third of hemodialysis patients.[3]

A reverse relationship between plasma serotonin and patients' age was detected. Considering that old age is associated with low-turnover bone disease in hemodialysis patients,[30,31] and that according to the results of the present study serotonin increases bone turnover, it seems possible that decreased with age circulating serotonin may contribute to the high incidence of low-turnover bone disease in elderly hemodialysis patients. Interestingly, β-CTx was also negatively related to patients' age and osteocalcin almost reach statistical significance regarding its negative relation to patients' age.

Diabetic hemodialysis patients are also characterized by increased incidence of low-turnover bone disease.[30,31] Compared to nondiabetic hemodialysis patients, osteocalcin, P1NP and β-CTx were much lower in diabetic hemodialysis patients. Serotonin was also lower in diabetic hemodialysis patients indicating once again that low peripheral serotonin may contribute to the high incidence of low-turnover bone disease in this group of hemodialysis patients. On the contrary, serum intact PTH level did not differ significantly between diabetic and non-diabetic hemodialysis patients, possibly because patients treated with vitamin D receptor activators or calcimimetics, who usually have the higher intact PTH values, were excluded from the study. However, and despite the high *P* value of .78 that was calculated for this comparison, a type 2 error due to the small number of the subjects cannot be excluded with certainty. Interestingly, serotonin level was significantly higher in nondiabetic hemodialysis patients than in healthy volunteers, whereas not such a difference was revealed between diabetic hemodialysis patients and healthy volunteers.

CONCLUSIONS

The present study adds to the relatively scarce clinical data about the effect of peripheral serotonin on bone metabolism. Certainly more studies are required for definitely clarifying the effect of peripheral serotonin on bone metabolism. However, according to the present study, it is possible that peripheral serotonin increases both bone formation and bone resorption in hemodialysis patients. The negative relation of serotonin to patients' age as well as its lower level in diabetic hemodialysis patients indicate that low plasma serotonin may contribute to the higher incidence of low-turnover bone disease that characterizes old and diabetic hemodialysis patients. The clinical impact of the above findings is very early to be predicted. However, better understanding of renal bone disease pathophysiology, certainly will help to improved treatment of this common complication.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Malluche HH, Langub MC, Monier-Faugere MC. The role of bone biopsy in clinical practice and research. Kidney Int Suppl. 1999;73:S20-5.
2. [No author listed]. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis. 2003;42:S1-201.
3. Qi Q, Monier-Faugere MC, Geng Z, Malluche HH. Predictive value of serum parathyroid hormone levels for bone turnover in patients on chronic maintenance dialysis. Am J Kidney Dis. 1995;26:622-31.
4. Jonnakuty C, Gragnoli C. What do we know about serotonin? J Cell Physiol. 2008;217:301-6.
5. Rosen CJ. Serotonin rising--the bone, brain, bowel connection. N Engl J Med. 2009;360:957-9.
6. Bliziotes M. Update in serotonin and bone. J Clin Endocrinol Metab. 2010;95:4124-32.
7. Kawai M, Rosen CJ. Minireview: A skeleton in serotonin's closet? Endocrinology. 2010;151:4103-8.
8. Ducy P, Karsenty G. The two faces of serotonin in bone biology. J Cell Biol. 2010;191:7-13.
9. Bliziotes MM, Eshleman AJ, Zhang XW, Wiren KM. Neurotransmitter action in osteoblasts: expression of a functional system for serotonin receptor activation and reuptake. Bone. 2001;29:477-86.
10. Westbroek I, van der Plas A, de Rooij KE, Klein-Nulend J, Nijweide PJ. Expression of serotonin receptors in bone. J Biol Chem. 2001;276:28961-8.
11. Battaglino R, Fu J, Spate U, et al. Serotonin regulates osteoclast differentiation through its transporter. J Bone Miner Res. 2004;19:1420-31.
12. Bliziotes M, Eshleman A, Burt-Pichat B, et al. Serotonin transporter and receptor expression in osteocytic MLO-Y4 cells. Bone. 2006;39:1313-21.
13. Gustafsson BI, Thommesen L, Stunes AK, et al. Serotonin and fluoxetine modulate bone cell function in vitro. J Cell Biochem. 2006;98:139-51.
14. Yadav VK, Ryu JH, Suda N, et al. Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. Cell. 2008;135:825-37.
15. Diem SJ, Blackwell TL, Stone KL, et al. Use of antidepressants and rates of hip bone loss in older women: the study of osteoporotic fractures. Arch Intern Med. 2007;167:1240-5.
16. Haney EM, Chan BK, Diem SJ, et al. Association of low bone mineral density with selective serotonin reuptake inhibitor use by older men. Arch Intern Med. 2007;167:1246-51.
17. Richards JB, Papaioannou A, Adachi JD, et al. Effect of selective serotonin reuptake inhibitors on the risk of fracture. Arch Intern Med. 2007;167:188-94.
18. van den Brand MW, Pouwels S, Samson MM, et al. Use of anti-depressants and the risk of fracture of the hip or femur. Osteoporos Int. 2009;20:1705-13.
19. Vestergaard P, Rejnmark L, Mosekilde L. Anxiolytics, sedatives, antidepressants, neuroleptics and the risk of fracture. Osteoporos Int. 2006;17:807-16.
20. Modder UI, Achenbach SJ, Amin S, Riggs BL, Melton LJ, 3rd, Khosla S. Relation of serum serotonin levels to bone density and structural parameters in women. J Bone Miner Res. 2010;25:415-22.
21. Takahashi M, Kushida K, Nagano A, Inoue T. Comparison of the analytical and clinical performance characteristics of an N-MID versus an intact osteocalcin immunoradiometric assay. Clin Chim Acta. 2000;294:67-76.
22. Ebeling PR, Peterson JM, Riggs BL. Utility of type I procollagen propeptide assays for assessing abnormalities in metabolic bone diseases. J Bone Miner Res. 1992;7:1243-50.
23. Okabe R, Nakatsuka K, Inaba M, et al. Clinical evaluation of the Elecsys beta-CrossLaps serum assay, a new assay for degradation products of type I collagen C-tlopeptides. Clin Chem. 2001;47:1410-4.
24. Coen G, Ballanti P, Bonucci E, et al. Bone markers in the diagnosis of low turnover osteodystrophy in haemodialysis patients. Nephrol Dial Transplant. 1998;13:2294-302.
25. Ueda M, Inaba M, Okuno S, et al. Clinical usefulness of the serum N-terminal propeptide of type I collagen as a marker of bone formation in hemodialysis patients. Am J Kidney Dis. 2002;40:802-9.
26. Okuno S, Inaba M, Kitatani K, Ishimura E, Yamakawa T, Nishizawa Y. Serum levels of C-terminal telopeptide of type I collagen: a useful new marker of cortical bone loss in hemodialysis patients. Osteoporos Int. 2005;16:501-9.
27. Tsuchida T, Ishimura E, Miki T, et al. The clinical significance of serum osteocalcin and N-terminal propeptide of type I collagen in predialysis patients with chronic renal failure. Osteoporos Int. 2005;16:172-9.
28. Rix M, Andreassen H, Eskildsen P, Langdahl B, Olgaard K. Bone mineral density and biochemical markers of bone turnover in patients with predialysis chronic renal failure. Kidney Int. 1999;56:1084-93.
29. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev. 2000;21:115-37.
30. Salusky IB, Goodman WG. Adynamic renal osteodystrophy: is there a problem? J Am Soc Nephrol. 2001;12:1978-85.
31. Andress DL. Adynamic bone in patients with chronic kidney disease. Kidney Int. 2008;73:1345-54.

Correspondence to:
Theodoros Eleftheriadis, MD, PhD,
Department of Nephrology, Medical School, University of Thessaly, Neo Ktirio, Mezourlo Hill, 41110 Larissa, Greece
Tel: +33 241 350 1668
E-mail: teleftheriadis@yahoo.com

Received January 2012
Revised August 2012
Accepted September 2012