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Tc-99m-HMDP bone uptake quantification and plasma osteocalcin levels in hemodialysis patients—a preliminary study

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In this preliminary study plasma osteocalcin levels and Tc-99m-HMDP (Technetium 99m hydroxymetylene diphosphonate) bone uptake (BU) were measured in 10 chronic end-stage renal failure patients who were on maintenance hemodialysis. The aim of this study was to determine the correlation between bone uptake and osteocalcin-a sensitive and specific marker of osteoblastic activity. There was a statistically significant increase in both 20 and 180 minute uptake in the patient group (36 ± 2.7 and 39 ± 3.6) when compared to the normal volunteers (32 ± 3.1 and 19 ± 2.7). Plasma osteocalcin levels were also significantly high (24.5 ± 5.6 ng/m*l*) when compared with normal values (6.5 ± 2.3 ng/m*l*). The correlations between osteocalcin and 20 and 180 min BU were high ($r=0.62$ and 0.72 respectively). In conclusion, our preliminary study suggests that, in hemodialysis patients, Tc-99m-HMDP bone uptake quantification is a sensitive and non-invasive method for showing increased osteoblastic activity.

Key words: osteocalcin, Tc-99m-HMDP bone uptake, hemodialysis patients

INTRODUCTION

IN CHRONIC end-stage renal disease, renal osteodystrophy (ROD) may result from secondary hyperparathyroidism, abnormalities in vitamin D metabolism, chronic metabolic acidosis and other complex mechanisms. According to bone histomorphometric studies, these patients are classified as slow and high turnover RODs, and it has been reported that plasma osteocalcin levels are consistently increased in both groups.[1] Plasma osteocalcin levels are reported to be a sensitive and spesific indicator of osteoblastic activity[2] and correlated well with histomorphometric parameters of bone formation.[3] However, there are controversial reports with respect to its correlation with parameters of bone mineralization.[4]

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BU quantification with Tc-99m labeled phosphate compounds have been used as a radionuclide diagnostic test in the evaluation of patients with metabolic bone disease. Increased bone uptake has been reported in patients with ROD probably due to increased osteoblastic activity. In these studies, several quantification methods, such as bone to soft-tissue activity ratios,[5–7] whole body radionuclide retention,[8] total skeletal activity,[9] and regional bone standard ratios[10] were used. However, in this study, BU was evaluated by using a different quantification method with three phase bone scan.

The aim of our study was to investigate the correlation between plasma osteocalcin levels and Tc99m-HMDP bone uptake values in end-stage renal disease patients who are on maintenance hemodialysis.

MATERIALS AND METHODS

Patients

10 end-stage renal failure patients (mean age: 34, range: 10–54 years; 7 males and 3 females) on

maintenance hemodialysis were studied. The mean durations of hemodialysis and end-stage renal failure were 8 months and 17 months respectively. Hemodialysis was performed twice weekly for approximately 5 hours with a cellulose membrane (Cuprophane) dialyser. The patients were on normal daily activities and received calcium carbonate and phosphate binders. Serum alkaline phosphatase, PTH-C and PTH-M levels of the patient group are shown in Table 1.

10 normal volunteers without any metabolic bone disease (mean age: 35, range: 18–65 years; 8 males and 2 females) were studied as the control group. Informed consent was obtained before study in all patients and normal volunteers.

RIA for osteocalcin

Blood samples for osteocalcin were taken just before hemodialysis and RIA for osteocalcin was performed with Human Osteocalcin RIA Kit OSTK-PR; CIS. The normal ranges were 6.6±2.3 ng/m*l* for men and 4.8±2.1 ng/m*l* for women. The sensitivity of the assay was 0.35 ng/m*l*. The intraassay cv was %4.7± 0.6 and interassay cv was %5.4±0.8.

Bone uptake quantification

Bone uptakes were quantified from the skull since there was almost no soft tissue attenuation of the gamma ray emission. After IV injection of Tc-99m-HMDP (550 MBq), 3 phases are recorded which represent the vascular, early and late bone uptakes. Lateral cranium views in a 64×64 matrix were recorded on disk for further processing of bone uptake. The acquisition times were: 1) From 0 to 120 seconds with one frame per second for the vascular phase; 2) At 20 and 180 minutes with static acquisitions of 1 minute for the early and late bone uptakes. Using ROI's of 4×4 pixels, initial vascular activity was measured in the torcular region during 60 seconds after the beginning of flow activity in the cerebral vessels. The bone activity was measured on the temporal region at 20 and 180 minutes and the background activity was taken on the neck region. Figure 1 illustrates the location of the ROI's. The bone uptake ratio was quantified as an index from the following formula:

Bone activity at time t—background activity/ vascular activity 0 to 60 sec—background activity.

Results are expressed as the mean±standard error. Students' unpaired t test was used for statistical analysis.

Table 1 Alkaline phosphatase (AP), PTH-C and PTH-M values in hemodialysis patients

No	AP (IU/m*l*)	PTH-C (pg/m*l*)	PTH-M (ng/m*l*)
1	156	918	855
2	183	710	680
3	476	128	92
4	165	135	62
5	214	137	80
6	173	229	105
7	112	163	120
8	240	150	110
9	147	419	390
10	95	116	95
Mean±SE	196±34	310±90	315±94
Normal range	41–133	20–90	0–27

RESULTS

The results of bone uptake quantifications in normal volunteers and in hemodialysis patients are shown in Figure 2. 20 min BUs were 32±3.1 and 36±2.7, and 180 min BUs were 19±2.7 and 39±3.6 in normal volunteers and hemodialysis patients respectively. The increase in BUs in hemodialysis patients was statistically significant ($p<0.05$). The osteocalcin levels and BUs of hemodialysis patients are shown in Table 2. The osteocalcin level in the patient group was 24.5±5.6 ng/m*l* which was significantly higher than the normal range 6.5±2.3 ng/m*l*. The correla-

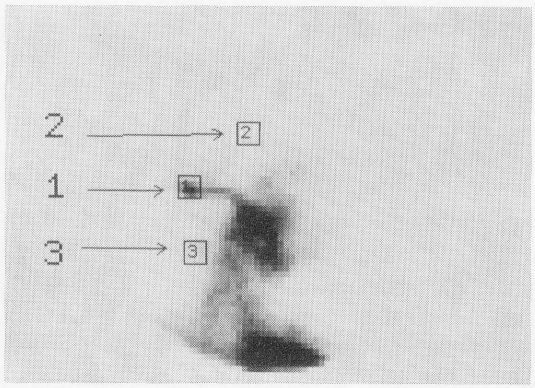

Fig. 1 Lateral cranium view shows locations of the ROIs in the vascular phase (1: Torcular, 2: Temporal, 3: Background regions).

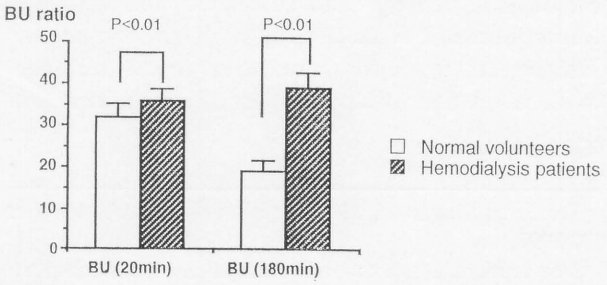

Fig. 2 Bone uptake (BU) ratio in normal volunteers and hemodialysis patients.

Table 2 Osteocalcin and bone uptake values in hemodialysis patients

No	BU (20 min)	BU (180 min)	Osteocalcin (ng/ml)
1	51	50	55.0
2	43	64	45.0
3	29	31	12.4
4	39	40	24.5
5	37	42	45.6
6	36	37	20.4
7	28	27	9.0
8	46	45	7.0
9	25	29	17.0
10	28	29	9.4
Mean±SE	36±2.7	39±3.6	24.5±5.6
Normal value	32±3.1	19±2.7	6.5±2.3

tion between osteocalcin and 20 min BU and 180 min BU was high (r: 0.62 and r: 0.72 respectively).

DISCUSSION

Bone uptake quantification has been proposed as a useful index in the assessment of patients with metabolic bone disease. De Graaf et al. have found that computerized quantitative analysis is an accurate scintigraphic method for detecting ROD, and that secondary hyperparathyroidism is the major cause of increased tracer uptake as judged from bone histomorphometric analysis.[9]

In this study, a simple quantification method by using three phase bone scintigraphy which minimizes soft tissue activity and enables shorter imaging time, was performed in order to evaluate early and late bone uptakes. Our results showed that both 20 and 180 minute uptakes were increased in hemodialysis patients who have ROD. Although the exact mechanism of Tc-99m-HMDP accumulation in bone is still not known, it is currently accepted that the selective concentration of the tracer by the hydroxyapatite crystals and chemisorbtion are responsible for it.[10] A large surface area to volume ratio and large hydration shells are some chemical and physieal properties of the hydroxyapatite crystal responsible for direct tracer incorporation.[11] However, there are other studies which indicate that immature collagen,[12] bone cells,[13] and enzymes or enzyme receptor sites in the organic matrix[14] may play a role in tracer uptake.

Osteocalcin (Bone GLA Protein) (BGP) is an extracellular protein with a molecular weight of 5700. It contains three residues of calcium-binding amino acid [gamma-carboxyglutamic acid (Gla)], and is synthesized specifically by the osteoblasts.[15] Several studies suggest that plasma osteocalcin levels reflect the portion of the newly synthesized protein which is not bound to the mineral phase of bone, but instead which is directly released into the circulation.[16] In ROD osteocalcin levels are uniformly increased as a result of both increased bone turnover and decreased renal filtration and degradation. However, numerous reports indicate that increased osteocalcin is highly correlated (r=0.72) to histomorphometric parameters of increased bone formation[17] and reflects increased activity of the osteoblast.[18]

Since bone biopsies are invasive in nature we tried to correlate BUs with osteocalcin levels. Our preliminary results show that in hemodialysis patients both osteocalcin levels and BUs are increased. Moreover, there was a high correlation between these 2 parameters. According to these results it can be hypothesized that increased osteocalcin might be involved in the binding of Tc-99m-HMDP to hydroxyapatite crystals; thereby increasing bone uptake in hemodialysis patients. Further studies are needed to elucidate this.

In conclusion, our preliminary study suggests that bone uptake quantification is a sensitive and noninvasive method for showing the increased osteoblastic activity in hemodialysis patients. Further studies are needed in this regard.

REFERENCES

1. Malluche HH, et al: The value of bone biopsies for diagnosis of renal bone disease. Dialysis Transplantation. *Nephrology*, pp 111–115, 1973
2. Sebert JL, et al: Plasma Bone-GLA Protein: Assessment of its clinical value as an index of bone turnover and bone formation in hemodialysis patients. *Proc EDTA-ERA* 22: 1218, 1985
3. Malluche HH, et al: Plasma levels of bone GLA-protein reflect bone formation in patients on chronic maintenance dialysis. *Kidney Int* 26: 869, 1984
4. Hauschka PV, Reid ML: Vitamin K dependence of a calcium binding protein containing gamma-carboxyglutamic acid in chicken bone. *J Biol Chem* 253: 9063, 1978
5. Rosenthall L, Kaye M: Technetium-99m-pyrophosphate kinetics and imaging in metabolic bone disease. *J Nucl Med* 16: 33, 1975
6. Wiegmann T, Rosenthall L, Kaye M: Technetium-99m-pyrophosphate bone scans in hyperparathyroidism. *J Nucl Med* 18: 231, 1977
7. Ling G, Nebel G, Dorr S: Die Bedeutung der Szintimetrie im Vergleich mit Histomorphometrie, Parathormon, Densitometrie und Rontgendiagnostik bei renaler Ostheopathie. *Fortschr Geb Rontgenstr Nuklearmed Erganzungsband* 136: 9, 1981
8. Fogelman I, Bessent RG, Turner JG, et al: The use of whole-body retention of Tc-99m diphosphonate in the diagnosis of metabolic bone disease. *J Nucl Med*

19: 270, 1978
9. De Graaf P, Pauwels EKJ, Vos PH, et al: Observations on computerized quantitative bone scintigraphy in renal osteodystrophy. *Eur J Nucl Med* 9: 419, 1984
10. Jones AG, Francis MD, Davis MA: Bone scanning: Radionuclidic reaction mechanisms. *Semin Nucl Med* 6: 3, 1976
11. Francis MD, Russel RGG, Fleisch H: Diphosphonates inhibition formation of calcium phosphate crystals *in vitro* and pathological calcification *in vivo*. *Science* 165: 1264, 1969
12. Rosenthall L, Kaye M: Observations on the mechanism of Tc99m-labeled phosphate complex uptake in metabolic bone disease. *Semin Nucl Med* 6: 59, 1976
13. Guillemart A, Besnard JC, LePape A: Skeletal uptake of pyrophosphate labeled with technetium-95m and technetium-96, as evaluated by autoradiography. *J Nucl Med* 19: 895, 1978
14. Zimmer AM, Isitman AT, Holmes RA: Enzymatic inhibition of diphosphonate: A proposed mechanism of tissue uptake. *J Nucl Med* 16: 352, 1975
15. Beresford JN, Gallagher JA, Poser JV, et al: Production of osteocalcin by human bone cells *in vitro*: Effects of 1,25(OH)2D3, 24,25(OH)2D3, parathyroid hormone, and glucocorticoids. *Metab Bone Dis Rel* 5: 229, 1984
16. Lian JB, Couttes MC, Canalis E: Studies of hormonal regulation of osteocalcin synthesis in cultured fetal rat calvaria. *J Biol Chem* 260: 8706, 1985
17. Brown JP, Delmas PD, Malaval L, et al: Serum bone Glaprotein: A specific marker for bone formation in postmenopausal osteoporosis. *Lancet* 1: 1091, 1984
18. Ismail F, Epstein S, Pacifici R, et al: Serum bone GLA protein (BGP) and other markers of bone mineral metabolism in postmenopausal osteoporosis. *Calcif Tissue Int* 39: 230, 1986