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PLASMA BONE-GLA PROTEIN: ASSESSMENT OF ITS CLINICAL VALUE AS AN INDEX OF BONE TURNOVER AND BONE FORMATION IN HAEMODIALYSIS PATIENTS

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Summary

Plasma bone-Gla Protein (BGP) was measured in 27 patients on chronic haemodialysis or haemofiltration. Individual values ranged from normal to 10 times the upper limit of the normal range. Plasma BGP correlated with the histomorphometric parameters of bone turnover and bone formation and with plasma PTH and alkaline phosphatase. When patients were classified according to their bone formation rates, plasma BGP allowed a better distinction between high and low bone formation rates than alkaline phosphatase or plasma PTH.

Introduction

Plasma bone-GLA Protein (BGP) is a newly recognized protein of low molecular weight which is synthesized in bone, probably by osteoblast cells [1]. Its value as a sensitive biochemical marker of bone formation has been demonstrated in osteoporosis and parathyroid disorders [2]. Since plasma BGP is metabolised in the kidney, its blood concentration is therefore influenced by renal failure. However, Malluche et al have recently shown that BGP allowed a clear cut distinction between low turnover osteomalacia and hyperparathyroidism in patients on chronic haemodialysis [3]. In the present study, the relationship between plasma BGP and the histomorphometric parameters of bone turnover and bone formation was evaluated in a non-selected group of patients undergoing chronic haemodialysis or haemofiltration, with no overt radiological bone disease. In addition, the relative values of plasma BGP, PTH and alkaline phosphatase for the prediction of the bone formation status was determined.

Patients and methods

Patients

The study comprised 27 patients (18 males, 9 females) with a mean ± SD age of 49±16 years, who had been on chronic haemodialysis or haemofiltration,

three times weekly for 28±15 months. These patients had no overt radiological bone disease and none of them had undergone parathyroidectomy. All the patients had normal hepatic function. The aluminium content was less than 0.3μmol/L in the dialysate and less than 0.6μmol/L in the substitution fluid for haemofiltration. Oral treatment consisted of calcium carbonate (3–12g/day) and aluminium hydroxide (3–9g/day).

Bone histomorphometry

All patients underwent a trans-iliac bone biopsy after double labelling with demethylchlortetracycline 600mg/day (2 days on, 12 days off, 4 days on).

Histomorphometric analysis was performed on four non-consecutive 5μm thick, undercalcified Goldner stained sections. Two unstained sections were used for fluorescence microscopy. The following parameters were measured: trabecular bone volume (TBV), expressed in percent total trabecular bone space, osteoid volume (OV), expressed in percent trabecular bone volume, osteoid surface (OS), percent trabecular bone surface, osteoblastic surface (OBL.S), defined as osteoid surface covered with plump osteoblasts, expressed in percent trabecular bone surface, active resorption surface (ARS) defined as Howship lacunae occupied by osteoclasts, expressed in percent trabecular bone surface, osteoclast count per mm^2 bone area (OCL), total labelled surface (TLS) defined as the extent of double labelled surfaces plus single labelled surfaced divided by two, expressed in percent trabecular surface, mineral appositional rate (MAR) expressed in μm/day. The bone formation rate (BFR) at tissue level was calculated as MAR x TLS and expressed in $\mu m^3/\mu m^2$/day. Normal values for static and dynamic parameters obtained from Bordier [4] and Melsen [5] are shown in Table II.

Biochemical methods

Plasma BGP was measured in a single assay by a radioimmunological method using rabbit antibody raised against purified calf BGP (Kit CEA, Saclay, France). The detection limit was 0.75ng/ml and intra-assay variation was less than 10 per cent. PTH was measured using an anti-serum directed against the 53–84 portion of the molecule [6]. Plasma aluminium was measured by inductively coupled plasma emission spectrometry [7]. Routine biochemistry including alkaline phosphatase determination was made with a Technicon autoanalyzer. Normal values of the biochemical parameters are shown in Table II.

Results

Correlation between bone histomorphometry and plasma BGP, alkaline phosphatase and PTH

As shown in Table I, BGP correlated best with the parameters of bone formation including osteoblastic surface, total labelled surface and bone formation rate which represents the amount of bone newly deposited per unit of trabecular

TABLE I. Correlations between bone histomorphometric parameters and plasma BGP, alkaline phosphatase and PTH

Histomorphometric parameters (1)	Correlation coefficient (r) with BGP	Alk. phosphatase	PTH
OV	0.46*	0.42	0.17
OS	0.36	0.27	0.008
OBL.S.	0.78***	0.68***	0.64***
ARS	0.54**	0.61***	0.58**
OCL	0.54**	0.51**	0.58**
TLS	0.75***	0.56**	0.44*
MAR (2)	0.47*	0.48*	0.49*
BFR (3)	0.76***	0.55**	0.51**

(1) See methods in text for the significance of parameters.

(2) Calculation made in 19 patients after exclusion of eight patients with no distinct double labels.

(3) Bone formation rate was assumed to be zero in patients with non-measurable MAR.

*$p<0.05$; **$p<0.01$; ***$p<0.001$

bone surface. BGP was also significantly correlated, but to a lesser extent, with resorption parameters. The same was true for alkaline phosphatase and PTH, but the correlation coefficients were lower.

In addition BGP correlated with alkaline phosphatase ($r=0.59$; $p<0.01$) and plasma PTH ($r=0.41$; $p<0.05$) but not with calcium, phosphate or aluminium.

Comparison of patients classified according to their bone formation rate

The patients were divided into two groups according to their bone formation rate: a group of 17 patients with normal or low bone formation rate, i.e. with bone formation rate $<0.089\mu m^3/\mu m^2/day$ which is the mean value of normal controls of Melsen [5]; a group of 10 patients with high bone formation rate.

None of these patients had florid osteomalacia since none had increased osteoid seam thickness and none had aluminium histochemical staining on trabecular bone surfaces.

Table II shows that the high bone formation rate group had higher osteoblastic surface and higher osteoclastic resorption, whereas both groups had the same increase of osteoid volumes and surfaces.

Plasma BGP ranged from 3.1 to 119.6ng/ml in the whole group of patients and the mean value was significantly higher in the group with high bone formation rate. PTH was also significantly higher in this group, but the significance of the difference was less than for BGP. Alkaline phosphatase was not significantly different in the two groups.

TABLE II. Comparison of histomorphometric and biochemical results (mean ± SE) between two groups of patients classified according to their bone formation rates (BFR)

	BFR		
	Low (n=17)	High (n=10)	Normal values (mean ± SD or range)
Histomorphometry			
TBC (%)	21.6±1.4	24.4±2.5	21±5
OV (%)	6.1±0.9	7.4±1	2.5±1
OS (%)	47.7±3.9	47.9±4.7	16±6
OBL. S (%)	4.6±0.9	18.7±2.8***	5±3
ARS (%)	3.1±0.7	9±1.2***	0.5±0.5
OCL/mm²	0.59±0.14	2.1±0.3***	0.2±0.1
TLS (%)	4.9±0.9	26±2.9***	14±1.3
Biochemistry			
Ca (mmol/L)	2.4±0.05	2.36±0.4	2.10–2.62
PO_4 (mmol/L)	1.8±0.1	1.97±0.14	0.8–1.45
Al (μmol/L)	2.01±0.57	1.41±0.64	<0.30
PTH (pg/ml)	176±30	305±45*	20–45
Alk. phosphatase (IU/L)	129±15	200±36	60–170
BGP (ng/ml)	14.8±3	58±13***	2–10

t statistics for two means: *p<0.05; ***p<0.001

TABLE III. Prediction of bone formation rate by BGP, alkaline phosphatase and PTH

	BGP	Alk. phosphatase	PTH
Threshold value	20ng/ml	170 IU/L	200pg/ml
Well classified patients (% total)	78	74	73
Yates corrected χ^2	6.39**	3.12	4.06*
Sensitivity (%)	80	60	80
Specificity (%)	77	82	68

*p<0.05; **p<0.01

Value of biological parameters to predict bone formation (Table III)

Threshold value for BGP, alkaline phosphatase and PTH was chosen in order to get the highest number of well classified patients in the two groups of normal or low bone formation rate and of high bone formation rate. A Yates corrected χ^2 was then calculated and showed that only BGP and PTH had a significant predictive value. BGP had the same sensitivity as PTH and a better specificity.

Alkaline phosphatase had no significant predictive value since the χ^2 was not significant.

Discussion

As previously reported by others in both haemodialysis patients [3] and in patients with other metabolic bone disorders [2], BGP was better correlated with the histomorphometric parameters of bone formation, than with that of bone resorption. This suggests that BGP is primarily a marker of new bone formation. Although the patients had no liver abnormality which could have influenced their blood level of alkaline phosphatase, alkaline phosphatase by itself did not allow us to differentiate the patients with low bone formation from those with high bone formation. In contrast, BGP was significantly higher in patients with high bone formation. Furthermore, BGP had the same sensitivity but a better specificity than plasma PTH to predict the bone formation status of these patients. In conclusion, BGP seems to be a better index of bone formation than alkaline phosphatase and plasma PTH in patients with terminal renal failure.

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