Running title: Biochemical indices of renal osteodystrophy in Malta

Biochemical indices of renal osteodystrophy in dialysis patients on the island of Malta

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Abstract

Objectives: Renal osteodystrophy (ROD) has never been studied on the small Mediterranean island of Malta, which has a largely inbred population. The genetic contribution to the pattern of renal osteodystrophy is being increasingly recognized. We were thus interested in studying indices of bone turnover in Maltese end-stage renal failure patients. *Materials and methods:* Sixty unselected patients, representing 65% of all patients undergoing dialysis in the island's renal unit, were prospectively investigated over a period of 5 months with respect to symptoms, calcium/phosphate chemistry, intact parathyroid hormone (iPTH) and bone alkaline phosphatase (bAP). Bone histomorphometry, which is the gold standard in the diagnosis of ROD, was not within the reach of our small unit. Biochemical markers may not be as sensitive and specific as bone biopsy for individual patient diagnosis of ROD sub-type but they can give a fairly good indication of the spectrum of bone turnover on a population basis. The optimum combination of biochemical marker cut-offs available from studies in the literature was then employed to estimate bone turnover. *Results:* The following biochemical picture emerged: 42% had iPTH <79.7 pg/ml (which cut-off has a reported specificity of 93.7% for low turnover bone disease), 45% had iPTH >100pg/ml and bAP >10ng/ml (which combined cut-off has a reported specificity of 100% for high turnover bone disease), while 13% could not be classified (ie had intermediate values). *Conclusions:* Based on biochemical data, the pattern of bone turnover seems to be comparable to the European average. Further in-depth study using bone histomorphometry is warranted.

Keywords

bone alkaline phosphatase

diagnosis

epidemiology

intact parathyroid hormone

Malta

renal osteodystrophy

Introduction

Renal osteodystrophy (ROD), an obligatory accompaniment of end-stage renal failure (ESRF), carries a significant contribution to morbidity and mortality. Depending on the activity of bone cells, ROD can be broadly divided into high-turnover or low-turnover categories. High turnover bone disease (HTBD) includes hyperparathyroid bone disease (HBD), of which osteitis fibrosa is the severe form, and mixed uraemic osteodystrophy (MO). Low-turnover states (LTBD) include adynamic bone disease (ABD), osteomalacia and aluminium-related bone disease [1].

The genetic contribution to variations in bone and mineral metabolism in ESRF patients is being increasingly recognized. We were interested in studying indices of bone turnover in ESRF patients on the small island of Malta since it has a largely inbred population and thus offers the opportunity to identify genetic susceptibility traits.

The golden standard in ROD diagnosis is bone histomorphometry [1]. However bone biopsies were impossible to perform in our setting due to the cost involved and the lack of local expertise. Although there is no proper substitute for bone biopsy, intact PTH (iPTH) and bone alkaline phosphatase (bAP) are the best surrogate markers to date. They have been shown to correlate well with histomorphometric parameters of bone formation and resorption [2, 3]. Various histomorphometric studies have investigated the potential of different cut-off values of these two parameters to diagnose specific patterns of abnormal bone turnover in ROD.

We therefore embarked on a biochemical study of bone turnover in our patients using iPTH and bAP.

Patients and Methods

60 ESRF patients undergoing either haemodialysis (HD) or chronic ambulatory peritoneal dialysis (CAPD) accepted to participate in the study. This represented 65% of the total number of patients on dialysis (n=92) on the Maltese islands. All dialyses are co-ordinated from one centre located in the hospital on the main island. Approval by the local Ethics Committee was obtained. The characteristics of the study population are shown in Table 1, including the ingestion of vitamin D analogues and calcium carbonate. One patient's compliance with vitamin D analogues was seriously doubted, so he was excluded from statistical analysis involving vitamin D analogue treatment. The calcium concentration of peritoneal dialysate in CAPD patients was not changed during the study period and was 1.75 mmol/L in 54% and 1.25 mmol/L in 46% of patients. The haemodialysate calcium concentration was 1.5 mmol/L in the vast majority of patients, with 1 mmol/L being used in a few patients as the need arose. Bicarbonate was used as the dialysis fluid buffer. None of our patients had ever received aluminium-containing phosphate binders. The dialysis water was treated by reverse osmosis and the dialysate concentration of aluminium was kept up to Association for Advancement of Medical Instrumentation standards (that is < 0.01 ppm). For the purpose of the study, patients were monitored for a 5 month period from August 1999 to December 1999. Calcium and phosphate were determined monthly in the case of HD patients and every 6 weeks in the case of CAPD patients. Midway through the study iPTH and bAP were assayed and the patients were assessed for signs and symptoms of ROD and questioned on the phosphorus content of their diet.

Serum biochemistry samples were analyzed in a single run to avoid inter-batch variation. iPTH and bAP samples were also analyzed together at the end of the study. iPTH was assayed using an N-terminal capture immunoradiometric assay (Gamma-BCT Intact PTH) from

ImmunoDiagnostics Systems (IDS) Limited, Tyne-and-Wear, UK. For the sake of comparison with cut-off values quoted in the literature which used a C-terminal capture immunoradiometric assay, we converted our values using the regression equation provided by the manufacturer. bAP activity was assayed using an immunoassay (Alkphase-B) from Metra Biosystems Inc, CA, USA. bAP activity (U/l) was converted to bAP (ng/ml) mass using Gomez et al's regression equation [4].

Patients were examined by the same clinician (I. Galea). A standard questionnaire was used to assess symptoms of ROD (bone pain, proximal muscle weakness, fractures and pruritus) using a linear visual analogue scale to quantify severity. Attention was paid to co-existing diseases which could give rise to similar symptoms, such as osteoarthritis, diabetic neuropathy, gout, Paget's disease, rheumatoid arthritis and connective tissue disorders. Symptoms were regarded as significant when unexplained by the patient's clinical picture. Compliance with low phosphate dietary advice was assessed by enquiring about the patients' intake of 10 phosphorus-rich food items (milk, cheese, cottage cheese, ice-cream, yoghurt, liver, fish, cereals, rock buns, cola drinks).

Data collection and statistical analysis was done using Excel 97 and SPSS 8.0 for Windows. Since the data was not normally distributed, non-parametric statistics were used (Mann-Whitney test and Spearman correlation coefficient). 95% confidence intervals were used throughout and significant evidence against the null hypothesis was taken at $p<0.05$ (2-tailed).

Results

Calcium

Most patients were normocalcaemic (78%) but a substantial proportion (20%) were hypercalcaemic. The reference range for calcium was 2.2-2.6 mmol/l; calcium was corrected for albumin using the following formula: corrected Ca = measured Ca + [0.02(40-albumin)] [5]. Calcium levels were relatively stable as shown by the coefficient of variation, which was <5% in 51% of patients. Serum calcium was positively correlated with vitamin D analogue treatment ($r=0.506$, $p<0.0005$) but not with calcium carbonate treatment.

Phosphate

Most patients were hyperphosphataemic (75%). The reference range for phosphate was 0.87-1.45 mmol/l. 50% of patients had phosphate levels higher than the range recommended by the National Kidney Foundation (NKF)'s K/DOQI 2003 guidelines ie between 1.13 to 1.78 mmol/l [6]. Levels fluctuated widely during the study as shown by the coefficient of variation, which was >15% in 52% of patients. There was no correlation with serum calcium.

Calcium-phosphate product

55% of patients had their calcium-phosphate (CaP) product in line with NKF K/DOQI recommendations ie below 4.44 $mmol^2/l^2$ (55 mg^2/dL^2) [6]; in 12% it was higher than 5.81 $mmol^2/l^2$ (72mg^2/dL^2), which has been shown to be significantly associated with increased mortality [7]. The CaP product fluctuated widely during the study as shown by the coefficient of variation, which was

>55% in 66% of patients. The CaP product was significantly correlated with phosphate ($r=0.914$, $p<0.0005$) but not calcium.

Intact PTH

The mean iPTH was 163 pg/ml with a standard deviation of 192 pg/ml. The normal range of iPTH values in subjects with normal renal function was 8-61 pg/ml. As expected most dialysis patients had abnormally high levels but only 22% of patients had their iPTH level kept within the currently desired range [6]. In 18% and 60% of patients, the iPTH was higher and lower than this optimum range respectively. iPTH values were significantly lower in diabetic patients, but did not bear any significant association with dialysis modality or vitamin D analogue treatment, even if controlled for diabetes (Table 2).

Bone alkaline phosphatase

Most bAP values (72%) were in the normal range (15-41 U/l for males and 12-31 U/L for females). The mean was 30 U/l with a standard deviation of 25 U/l. BAP values were significantly lower in CAPD patients and in those on vitamin D analogue treatment, but did not bear any significant relationship with diabetes, the latter even if controlled for the other significant factors (Table 2).

Dietary phosphate

Although the patients' total phosphorus intake was not followed, their compliance with low phosphate dietary advice was assessed by inquiring about their intake of ten high phosphorus food items. These ten food items were amongst the food items they were routinely advised to avoid in clinic. Patients were ingesting a mean of 2.1 portions, equivalent to 222mg phosphorus, of these

restricted high phosphorus food items daily (standard deviations of 1.5 portions and 155mg phosphorus respectively). Thus dietary control of phosphate was not optimal.

Symptoms

Unexplained bone pain, fractures since diagnosis of renal disease, proximal muscle weakness and itching were present in 39%, 18%, 69% and 56% of patients respectively. The prevalence of unexplained bone pain was similar to that in a Toronto study [8] but more fractures (1.8 fold) and proximal muscle weakness (1.4 fold) were observed in our sample. None of the symptoms correlated significantly with any biochemical parameter (calcium, phosphate, iPTH, bAP) or differed significantly between dialysis modalities.

Bone turnover in the Maltese ESRF population

Bone biopsy was not possible due to a variety of reasons, foremost amongst which was the lack of funding. However we chose to assay iPTH and bAP since they are the best biochemical indicators of bone turnover to date. Since we were ready to sacrifice sensitivity for specificity, we trawled the literature for the combination of iPTH and bAP cut-off values with the maximum specificity and best diagnostic yield. Those of Fletcher et al (iPTH >100 pg/ml and bAP >10 ng/ml for high turnover bone disease) [9] and Coen et al (iPTH <79.7 pg/ml for low turnover bone disease) [10] were viewed to be the best available at the time with reported specificities of 100% for HTBD and 93.7% for LTBD. Since we used different assays from the ones used in these studies, we converted our data using validated regression equations as described under “Patients and Methods”. The following biochemical picture emerged: 42% had iPTH <79.7 pg/ml (suggestive of LTBD), 45% had iPTH >100pg/ml and bAP >10ng/ml (suggestive of HTBD), while 13% could not be

classified (ie had intermediate values) (Table 3). The biochemical markers of CAPD and HD patients were differentially skewed toward LTBD and HTBD respectively (Table 3).

Discussion

Malta is an archipelago of 3 islands in the Mediterranean, 60 miles south of Sicily. It has a combined area of 122 square miles and a population of about 400,000 people. The strongest genetic influences are Sicilian and Arabic. It is thought to have been initially populated by Sicilians in 5200 BC and retained links with Italy till it was invaded by the Phoenicians in 700 BC. It then passed through the hands of the Carthaginians, Romans, Arabs, Normans, Spaniards, Knights of St. John, French and English. Of these, the last strongest genetic influence has probably been the Arab invasion, lead by Habasa in AD 869–870, since the Maltese speak a Semitic language and generally have Semitic surnames, though the calligraphy is Roman. It has been a relatively inbred population probably since the Arabs left Malta in AD 1091. Although all other aspects of Maltese life are nowadays European, there is good reason to suspect that the genetic make-up of the Maltese differs significantly from that of the rest of Europe. Malta has strikingly different prevalence rates for certain diseases with a genetic predisposition compared to neighbouring European countries, for example low prevalence rates of multiple sclerosis [11] and the highest prevalence rates of diabetes in Europe (International Diabetes Federation statistics for 2002 at www.idf.org). This leads one to suspect that inbreeding of an island population might have given rise to a unique genetic make-up reflecting unique disease susceptibility characteristics.

Polymorphisms at several different genetic loci have been implicated in renal osteodystrophy. The common Bsm1 vitamin D receptor polymorphism can result in BB, Bb or bb

genotypes. The bb genotype is associated with hyperparathyroidism in ESRF patients as opposed to Bb or BB, and it may also be protective against ABD [12]. Interestingly the bb genotype is associated with higher bone mineral density [13] and Malta has a notably low incidence of osteoporotic fractures [14, 15]. This polymorphism is important since the BB genotype exhibits higher sensitivity to vitamin D analogues [16]. Other candidate polymorphisms are the calcium-sensing receptor [17] and parathyroid hormone [18].

With this background in mind we were interested to know how the pattern of bone turnover in Maltese dialysis patients compares with elsewhere. There is one renal unit on the main island which was serving 92 patients at the time of the study. We prospectively followed 60 of these patients over 6 months looking at biochemical indices of bone turnover. Despite being the gold standard for diagnosis, bone biopsy was not within the reach of a small renal unit such as ours.

There was a high prevalence of hyperphosphataemia amongst our dialysis population (75%), with a substantial proportion (28%) having a mean phosphate of >2.1mmol/l during the study, which has been found to carry a relative risk of mortality of 1.27 [7]. Compliance with dietary phosphate restriction was poor. Calcium was relatively well controlled. The CaP product exceeded NKF K/DOQI recommendations in 45% of patients; it fluctuated mostly as a function of phosphate. Vitamin D analogue doses were difficult to titrate properly because of the lack of availability of regular PTH monitoring, the only guide being the calcium level.

Intact PTH and bAP were selected since they are the best markers of bone turnover to date. Results confirmed the known risk factors for LTBD: peritoneal dialysis, diabetes and vitamin D analogue treatment (Table 2). Furthermore our CAPD patients were probably at higher risk of relative hypoparathyroidism since 54% of them were dialysed against a calcium concentration of 1.75 mmol/l. It is now known that when such patients are converted to a lower calcium-containing dialysate (1.25 mmol/l), iPTH levels increase [19, 20].

We classified patients into high and low bone turnover groups using iPTH and bAP cut-off values which have been standardized against bone histomorphometry in previous studies. We chose those cut-offs which maximized specificity to favour case ascertainment. We recognize that this approach is not standard but it was the best we could do in the circumstances in order to get a glimpse of bone turnover in our study population. 45% and 42% of patients had a biochemical picture compatible with HTBD and LTBD respectively, while the picture remains unknown in 13% since their results could not be classified using the chosen cut-offs. We believe that no LTBD in our population is aluminium-induced since our unit pursues an aggressive aluminium-low policy, though plasma aluminium levels were not measured in the study. Given that 13% could not be classified, the true prevalence of LTBD could be anything between 42-55%, though it's more likely to be closer to the lower value due to mixed osteodystrophy patients and the false positives arising from the 93.7% specificity in diagnosis. These prevalences are comparable with those in Europe, Canada and USA. In a pan-European study, the prevalences of LTBD and HTBD were 47% and 41% respectively [21], with similar results from Canada [22] and USA [23]. All these studies used bone histomorphometry to establish diagnosis. Interestingly, prevalence of ABD in neighbouring Italy has been consistently reported to be low, in the range of 14-22% [10, 24, 25].

In summary, we have presented here the first biochemical study of bone turnover in dialysis patients on the island of Malta. The patterns observed do not differ drastically from the European average, though no firm conclusions can be reached until further in-depth study using bone histomorphometry is carried out.

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Tables

Table 1. Patient demographics at time of entry into study and ingestion of vitamin D analogues and calcium carbonate. SD = standard deviation

Clinical characteristics	HD	CAPD
Number of patients	36	24
Sex (*male/female*)	22/14	15/9
Age (*years ± SD)*	54.7 ±14	56.8 ±15
Dialysis duration (*months ± SD*)		
➢ total	20.9 ±16.4	19.1 ±15.2
➢ since last modality switch	17 ±14.3	18.3 ±14.6
Diabetes mellitus (%)	33.3	16.7
% patients on vitamin D analogues	40	54
% patients on $CaCO_3$	89	100

Table 2. Mean iPTH (pg/ml) and bAP (U/l) values (mean ± standard deviation) according to dialysis modality, diabetic status and vitamin D analogue treatment

	CAPD	HD	*p* value
	n=24	n=36	
PTH	139.5 ± 164.7	162.9 ± 178.5	0.551
bAP	20.3 ± 11.23	37.2 ± 29.8	<0.0001
	Diabetic	Non-diabetic	*p* value
	n=16	n=44	
PTH	58.7 ± 57.7	188.1 ± 186.8	0.002
bAP	21.9 ± 9.7	33.6 ± 28.6	0.132
	On vitamin D analogues	Not on vitamin D analogues	*p* value
	n=27	n=32	
PTH	119.5 ± 120.3	181.4 ± 205.9	0.181
bAP	25 ± 16.2	35.5 ± 30.8	0.05

Table 3. Biochemical assessment of bone turnover using iPTH and bAP cut-offs as described in the text. HTBD = high turnover bone disease. LTBD = low turnover bone disease.

	Biochemical picture of		
	HTBD	LTBD	Unclassified
All patients n=60	45%	42%	13%
CAPD n=24	33%	50%	17%
HD n=36	53%	36%	11%