Serum 1,25 dihydroxyvitamin D and osteocalcin concentrations in thalassaemia major

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SUMMARY In view of the claim that low 25-hydroxyvitamin D (25-OHD) concentrations may contribute to the pathogenesis of bone disease in patients with β thalassaemia major and iron overload, we have assessed the concentrations of 25-OHD, $1\alpha,25$ dihydroxyvitamin D ($1\alpha,25(\text{OH})_2\text{D}$), parathyroid hormone, and osteocalcin in such patients. 25-OHD concentrations were significantly lower in patients with thalassaemia major and iron overload than in controls and in some patients were subnormal or undetectable. $1\alpha,25(\text{OH})_2\text{D}$ concentrations were, however, normal in all patients and were similar to those in controls. Serum parathyroid hormone and plasma calcium concentrations were also normal and not significantly different from those in controls. Although 25-OHD concentrations increased significantly between January and June, there was no change in $1\alpha,25(\text{OH})_2\text{D}$ concentrations. 25-OHD concentrations remained lower than control values, even in June. Parathyroid hormone concentrations fell, but not significantly, between January and June, but calcium concentrations did not alter. Osteocalcin concentrations were normal in all patients except one, who had extremely low concentrations of this protein. The concentration of osteocalcin was not related to 25-OHD or $1\alpha,25(\text{OH})_2\text{D}$ concentrations. Thus normal calcium homeostasis is maintained in patients with thalassaemia major despite low or low-normal 25-OHD concentrations; this is probably achieved through the maintenance of normal $1\alpha,25(\text{OH})_2\text{D}$ concentrations, which were indistinguishable from those in controls. Normal $1\alpha,25(\text{OH})_2\text{D}$, parathyroid hormone, and osteocalcin concentrations argue against an important role for vitamin D deficiency in the pathogenesis of thalassaemic bone disease.

Previous studies on the vitamin D state of patients with β thalassaemia major and iron overload have shown that although these patients have no abnormalities in their plasma calcium and phosphate concentrations, the concentrations of 25-hydroxyvitamin D (25-OHD) are often low and are subject to seasonal variations.^{1,2} It has been suggested by some authors that this marginal deficiency of 25-OHD may contribute to bone disease in patients with β thalassaemia major and iron overload.³ Bone disease in this condition may be severe enough to cause pathological fractures.³ It is surprising therefore that no study investigating concentrations of $1\alpha,25$ dihydroxyvitamin D ($1\alpha,25(\text{OH})_2\text{D}$) has hitherto been published. This is particularly important as parathyroid hormone deficiency is also known to occur in patients with β thalassaemia major and severe iron overload,⁴ and parathyroid hormone regulates the renal hydroxyla-

tion of 25-OHD to $1\alpha,25(\text{OH})_2\text{D}$. We therefore undertook a study to determine (1) whether $1\alpha,25(\text{OH})_2\text{D}$ concentrations were responsible for the normal and relatively constant calcium concentrations in these patients and (2) whether $1\alpha,25(\text{OH})_2\text{D}$ concentrations altered in these patients with the change in seasons. We also undertook measurements of serum osteocalcin, an index of osteoblastic activity, in these patients.

Patients and methods

A series of 15 patients (seven male and eight female, age range 18–28 years) with thalassaemia major were investigated. All patients had severe iron overload. Serum ferritin concentration ranged between 900 and 932 $\mu\text{g/l}$. The patients had been treated with chelation therapy with subcutaneous desferrioxamine infusions. They were all fairly well,

mobile, and active and were having blood transfusions at four weekly intervals. Aspartate transaminase activities ranged between 45 and 225 U/l (normal range 5–35 U/l). All patients had blood samples collected in January, and seven of them were reinvestigated in June of the same year.

Plasma calcium, phosphate, and albumin concentrations and alkaline phosphatase activity were measured using a SMAC Technicon Autoanalyser. Serum parathyroid hormone concentration was measured by a radioimmunoassay using an antibody directed against the mid-fragment of the parathyroid hormone molecule.⁴ Serum 25-OHD concentration was measured by a technique modified from Preece *et al*⁵ and serum $1\alpha,25(\text{OH})_2\text{D}$ concentration by the technique of Reinhardt *et al*⁶ Osteocalcin in serum was measured by a specific radioimmunoassay as described by Deftos *et al*.⁷ (The reagents for these assays (parathyroid hormone, $1\alpha,25(\text{OH})_2\text{D}$, and osteocalcin) were obtained from Immunonuclear Corporation, Stillwater, Minnesota, United States.) The details of the sensitivity and precision of these assays have been published before.⁸

Statistical analysis was carried out using Student's *t* test.

Results

Calcium, phosphate, and albumin concentrations were within the normal range in all patients and were not different from those in controls (Table 1).

Serum 25-OHD concentrations of the 15 patients

studied in January (mean(SD) 12.6(6.7)nmol/l) were significantly lower than control values (mean(SD) 35(10)nmol/l) (Table 2). Ten of 18 patients studied in January had subnormal 25-OHD concentrations. The serum 25-OHD concentration was greater in June than in January in each of the seven patients in whom such paired samples were available. There were no corresponding increases in calcium concentrations (Table 1). The concentration of 25-OHD in these patients was also lower than that in controls during the summer, but this difference was not significant.

$1\alpha,25(\text{OH})_2\text{D}$ concentrations were in the middle of the normal range of all patients, irrespective of concomitant subnormal or low-normal 25-OHD concentrations. There was no significant change in $1\alpha,25(\text{OH})_2\text{D}$ concentrations between January and June (Table 2).

Parathyroid hormone concentrations were within the normal range in all patients; they fell by a small but not significant amount between January and June (Table 2). A similar small diminution in parathyroid hormone was also observed in the controls. Parathyroid hormone concentrations in patients with thalassaemia were lower than those in controls in both summer and winter, but this difference was not significant.

Osteocalcin concentrations ranged between 0.3 and 11.7 ng/ml (mean(SD) 5.3(2.2)ng/ml); in one patient it was subnormal (0.3 ng/ml). Osteocalcin concentrations were not related to 25-OHD, $1\alpha,25(\text{OH})_2\text{D}$, or parathyroid hormone concentrations.

Table 1 Calcium and phosphate concentrations and alkaline phosphatase activity in patients with thalassaemia major and controls. Values are mean (SD) [range]

	Calcium (nmol/l)	Phosphate (nmol/l)	Alkaline phosphatase (IU/l)
Patients with thalassaemia (winter)	2.43 (0.12) [2.25–2.55]	1.0 (0.12) [0.9–1.15]	105 (15) [86–130]
Controls	2.41 (0.13) [2.21–2.56]	1.1 (0.15) [0.85–1.18]	101 (16) [83–125]

Table 2 Serum 25-hydroxyvitamin D (25-OHD) $1\alpha,25$ dihydroxyvitamin D ($1\alpha,25(\text{OH})_2\text{D}$), and parathyroid hormone concentrations in patients with thalassaemia and controls: effect of seasons. Values are mean (SD)

	25-OHD (nmol/l)	$1\alpha,25(\text{OH})_2\text{D}$ (pmol/l)	Parathyroid hormone (pmol/l)
Patients with thalassaemia:			
Summer (n=7)	28 (12.1)**	86.1 (10.9)	36.4 (9.1)
Winter (n=15)	12.6 (6.7)*	88.9 (11.1)	40 (19)
Controls:			
Winter	35 (10)	85 (20)	55 (15)
Summer	40 (12)	81 (19)	48 (16)

* $p < 0.002$ when compared with controls in winter and $p < 0.01$ themselves in summer.

** $p < 0.05$ when compared with controls in summer.

Discussion

These data show that although serum 25-OHD concentrations are low-normal or subnormal in patients with β thalassaemia major, their serum $1\alpha,25(\text{OH})_2\text{D}$ concentrations are in the middle of the normal range. Furthermore, while pronounced seasonal variations of 25-OHD concentrations occur, there is no seasonal alteration in $1\alpha,25(\text{OH})_2\text{D}$ concentrations. Plasma calcium concentrations also remained essentially unaltered. This is consistent with the fact that serum parathyroid hormone concentrations were within the normal range and did not alter significantly in these patients between January and June. It is noteworthy that significant vitamin D deficiency is associated with a rise in parathyroid hormone concentrations. We have previously shown this among Asians in the United Kingdom⁹ and in the elderly.⁸

These data indicate that calcium homeostasis is normal in patients with thalassaemia major, although the relative lack of sunshine in the UK contributes to diminished vitamin D stores as reflected by low or low-normal 25-OHD concentrations. The deficiency is more pronounced in winter. There is, however, sufficient 25-OHD to provide an adequate substrate for 1α -hydroxylation by the kidney, and therefore these patients have normal concentrations of $1\alpha,25(\text{OH})_2\text{D}$, even during the winter months. It is relevant to mention that administration of vitamin D to patients with osteomalacia who have low 25-OHD and $1\alpha,25(\text{OH})_2\text{D}$ concentrations can restore $1\alpha,25(\text{OH})_2\text{D}$ concentrations to normal very rapidly¹⁰ and that minute quantities of calciferol are sufficient to generate large quantities of $1\alpha,25(\text{OH})_2\text{D}$. 25-OHD concentrations in serum are expressed in nmol/l, while those of $1\alpha,25(\text{OH})_2\text{D}$ are expressed in pmol/l.

Serum osteocalcin concentrations are thought to be markers for osteoblastic activity.^{11 12} As osteocalcin concentrations were normal in all patients except one it would seem that osteocalcin is not altered in most of these patients. This does not rule out the possibility, however, that the ratio of carboxylated to non-carboxylated osteocalcin may be altered in such patients. We have recently shown that carboxylation of osteocalcin in man is a vitamin K dependent process¹³ and that it may be altered in patients with primary biliary cirrhosis.¹⁴

Thus these data suggest that vitamin D deficiency probably does not contribute to the skeletal abnormalities observed in most patients with thalassaemia major. These abnormalities are probably due to the pronounced and diffuse extramedullary haemopoiesis and possibly the deposition of iron in skeletal

tissue known to occur in this condition.¹⁵ Whether the deposition of iron actively interferes with ossification is not known. Bone biopsy examinations have shown evidence of abnormal bone formation, but osteomalacia is extremely rare.¹⁵ There are reports, however, of the presence of thickened osteoid seams, without a lining of osteoblasts on these seams, in patients with β thalassaemia with severe untreated anaemia.¹⁶ This occurrence was associated with normal serum 25-OHD concentrations. In contrast, in a rare case of β thalassaemia without anaemia a high turnover of bone was observed with normal osteoblasts around osteoid seams.¹⁷ Thus anaemia itself may induce changes in the bone. Longstanding thalassaemia is associated with osteopenia and pathological fractures³—this could reflect cumulative effects of defective bone formation through various mechanisms.

Another factor contributing to osteopenia and pathological fractures in this condition could be the delay in the onset, or total absence of puberty. Hypo-oestrogenism associated with amenorrhoea and delayed puberty have recently been incriminated in the pathogenesis of osteopenia in anorexia nervosa,¹⁸ in women athletes,¹⁹ and in ballet dancers.²⁰ Finally, treatment of anaemia per se has been shown to correct histological abnormalities in patients with β thalassaemia major. Wide osteoid seams with few osteoblasts before treatment with transfusion were replaced by narrower osteoid seams and a pronounced increase in osteoblasts.¹⁸ There was also an increase in serum alkaline phosphatase activity.

In conclusion, the low or low-normal 25-OHD concentrations in patients with β thalassaemia major do not reflect pronounced abnormalities in vitamin D or calcium homeostasis. Nor is there any evidence of parathyroid hormone or calcium abnormalities in most patients with this condition. The skeletal abnormalities of thalassaemia are probably due to other mechanisms, and vitamin D supplementation is not likely to cause their resolution.

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