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Clinical, biochemical, and histological studies of osteomalacia, osteoporosis, and parathyroid function in chronic liver disease

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SUMMARY Twenty of 32 patients with either chronic cholestatic or hepatocellular liver disease had bone pain or recent fractures. On bone biopsy five patients had normal bone, 15 had osteomalacia, five had osteoporosis, and seven had a combination of osteomalacia and osteoporosis. In the presence of osteoporosis, osteomalacia was minimal or absent. There was no biochemical, radiological, or histological evidence of excess parathyroid activity. No significant correlations were demonstrated between the plasma and urinary biochemical findings and the presence of either osteoporosis or osteomalacia and bone biopsy was essential for correct diagnosis. There was no statistical relationship between low serum 25-hydroxy vitamin D values and the presence of osteomalacia. Bone disease was not prevented by regular intramuscular vitamin D_2, although biochemical changes were improved. Drugs such as corticosteroids and cholestyramine may be important aetiological factors in hepatic osteodystrophy.

Bone thinning and fractures are recognised complications of chronic cholestatic liver disease. Patients with either chronic cholestatis or hepatocellular liver disease may show histological evidence by bone biopsy of either osteomalacia or osteoporosis, and in some patients a combination of both (Atkinson *et al.*, 1956). Calcium malabsorption has been demonstrated in primary biliary cirrhosis (PBC) and in hepatocellular disease; this has been attributed to decreased intestinal absorption of vitamin D (Keyhayoglou *et al.*, 1968; Whelton *et al.*, 1971). In these patients there are low serum 25-hydroxy vitamin D (25-OHD) values (Long *et al.*, 1976) which are not usually due to a failure of hepatic 25-hydroxylation (Skinner *et al.*, 1977). Regular parenteral vitamin D_2 supplements have previously been shown to improve ^{47}Ca absorption in PBC but not to prevent bone disease (Kehayoglou *et al.*, 1968). Hypophosphataemia is a feature of chronic liver disease and this could be caused by an associated secondary hyperparathyroidism (Loeper *et al.*, 1939; Rochman, 1976). The present study was undertaken to assess the incidence and type of metabolic bone disease in patients with either cholestatic or hepatocellular liver disease, to correlate the bone histology with the biochemical data, and to determine if the latter could predict bone histology.

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Methods

Biochemical investigations were done after four-to-five days of stabilisation on a 20 mmol (800 mg) per day calcium diet. Venous blood samples were collected without stasis after a 12 hour overnight fast. Plasma concentrations of calcium, phosphorus, magnesium, alkaline phosphatase activity, total protein, albumin, creatinine and ethanol-extractable hydroxyproline were estimated by standard methods (Varghese *et al.*, 1973). The measured plasma calcium was corrected for variations in albumin concentration by the formula: adjusted calcium = (plasma calcium − albumin (g/dl) + 4) mg/dl (Payne *et al.*, 1973). This result was divided by 4 to obtain mmol/l. Alkaline phosphatase isoenzymes were estimated by heat inactivation (Whitby and Moss, 1975). Serum 25-hydroxy-vitamin D (25-OHD)

Table 1 *Clinical details (mean ± SD)*

	Total	*Cholestatic patients*	*Hepatocellular patients*
Number	32	25	7
Age (yr)	53·1 ± 10·7	52·9 ± 11·3	54·0 ± 8·7
Sex (M:F)	8:24	5:20	3:4
Duration of symptoms (yr)	4·7 ± 3·8	5·4 ± 4·0	2·1 ± 0·7
Years serum total bilirubin > than 51 μmol/l (3 mg/100 ml)	1·2 ± 1·4	1·4 ± 1·5	0·4 ± 0·8
Bone pain or fractures	20	17	3

concentration was measured by a modified competitive protein-binding technique (Long *et al.*, 1976) and immunoreactive-parathyroid hormone (i-PTH) by radioimmunoassay (Wills *et al.*, 1974). Two consecutive 24 hour urine collections were assayed for calcium, hydroxyproline and creatinine excretion and the mean of the two results used.

Vertical iliac crest bone biopsies were taken from all the patients under local anaesthesia with a Burkhardt drill. Specimens were processed by the method of Tripp and McKay (1972). Longitudinal sections were stained by standard haemotoxylin and eosin, Van Gieson, and reticulin techniques. After qualitative microscopy, quantatitive histological examination was undertaken using a rapid simple computerised technique (Meinhard *et al.*, 1975). The microscope image of the trabecular portion of the section was projected (×100 magnification) and traced directly onto computer data cards. An Optical Mark Reader fed the data into a minicomputer to provide tissue volume proportion (Vv%) of osteoid, mineralised bone and total bone, and surface extent (Sv%) of osteoblasts and osteoclasts. The normal range for increased osteoid tissue is less than 0·5% Vv and for total bone is more than 20%Vv; values above and below these values respectively indicate osteomalacia and osteoporosis.

Statistical method was by calculating product-moment correlation coefficients and by Student's *t* test. A multiple regression of increase in osteoid against total bone, plasma calcium, and phosphorus was also performed. Results are expressed as mean ± SD.

PATIENTS STUDIED (Table 1)

The 32 patients, 24 women and eight men, aged 21 to 64 years, had all been admitted to the Liver Unit at the Royal Free Hospital and diagnosis made on the basis of full investigation including liver biopsy. Twenty-five patients had cholestatic liver disease: PBC (20), secondary biliary cirrhosis (two), sclerosing cholangitis (one), postoperative biliary stricture (one), and childhood intrahepatic biliary atresia (one). Nineteen of the patients with PBC had clinical, biochemical, and histological evidence of late disease; in one the hepatic lesion was early. Seven patients had hepatocellular disease: alcoholic cirrhosis (three) alcoholic hepatitis without cirrhosis (three), and cryptogenic cirrhosis (one).

Duration of liver symptoms from onset to the time of bone biopsy was recorded together with the number of years that the serum total bilirubin had exceeded 51 μmol/l (3 mg/100 ml). The duration of symptoms for the patients with postoperative biliary disease was taken from the time of the first operation. The duration of liver symptoms ranged from four months to 18 years for the cholestatic patients. It was harder to assess duration of symptoms in the hepatocellular patients because the alcoholic patients tended to deny symptoms; it was probably about two to three years. In the cholestatic group the serum total bilirubin had exceeded 51 μmol/l (3 mg/100 ml) for 1·4 ± 1·5 years (eight of the 25 patients had never been jaundiced); in the hepatocellular group the mean years with a serum total bilirubin greater than 51 μmol/l was 0·4 ± 0·8 years.

Twenty of the patients complained of diffuse bone pain and eight of these had radiological evidence of recent fractures of hips, ribs, fibulae, or lumbar vertebrae; these had followed relatively minor trauma. Seventeen of the cholestatic patients (14 with PBC, two with secondary biliary cirrhosis, and one with sclerosing cholangitis) and three of the hepatocellular patients (two with alcoholic cirrhosis and one with alcoholic hepatitis) had either bone pain or fractures. Only one of the 32 patients (a case of PBC) showed radiological periosteal reactions on the lower end of the tibia. Pseudofractures were never detected.

Vitamin D_2 (ergocalciferol) 100 000 IU in ethyl oleate intramuscular per month, had been given regularly to 16 of the cholestatic patients (PBC, 14; secondary biliary cirrhosis, one; and biliary atresia, one) with a mean duration of treatment of 2·3 ± 2·1 (range one to six years). At the time of investigation, 10 patients had received cholestyramine, three prednisolone, three frusemide, and one phenobarbitone for more than six months.

Results

BIOCHEMICAL FINDINGS (Table 2)

In eight patients (PBC, five; biliary atresia, one; alcoholic cirrhosis, one; and cryptogenic cirrhosis,

Table 2 *Biochemical results (mean ± SD)*

	Total	*Cholestatic patients*	*Hepatocellular patients*	*Normal range*
Number	32	25	7	
Plasma:				
calcium mmol/l	2·28 ± 0·18	2·28 ± 0·18	2·23 ± 0·20	2·13-2·63
(mg/dl)	(9·1 ± 0·7)	(9·1 ± 0·7)	(8·9 ± 0·8)	(8·5-10·5)
phosphorus mmol/l	1·03 ± 0·16	1·00 ± 0·16	1·16 ± 0·19	0·87-1·45
(mg/dl)	(3·2 ± 0·5)	(3·1 ± 0·5)	(3·6 ± 0·6)	(2·7-4·5)
magnesium mmol/l	0·86 ± 0·10	0·90 ± 0·08	0·78 ± 0·25	0·74-0·99
(mg/dl)	(2·1 ± 0·3)	(2·2 ± 0·2)	(1·9 ± 0·6)	(1·8-2·4)
total protein g/l	79 ± 10	80 ± 11	76 ± 4	62-80
albumin g/l	36 ± 7	37 ± 7	36 ± 5	35-50
alkaline phosphatase K.A. units/dl	50 ± 36	60 ± 36	17 ± 8	3-13
hydroxy proline μmol/l	12·2 ± 6·1*	12·2 ± 6·1*	12·2 ± 7·6*	6·8-21·3
(mg/l)	(1·6 ± 0·8)	(1·6 ± 0·8)	(1·6 ± 1·0)	(0·9-2·8)
Serum				
25-OHD ng/ml	11·8 ± 11·5	13·7 ± 12·3	5·1 ± 3·9	9-44
i-PTH ng/ml	0·31 ± 0·07	0·32 ± 0·07	0·29 ± 0·06	0·25-0·95
24 h urinary				
calcium mmol	2·28 ± 1·33	2·45 ± 1·35	1·55 ± 0·98	25-75
(mg)	(91 ± 53)	(98 ± 54)	(62 ± 39)	(100-300)
hydroxy proline μmol	175 ± 85*	180 ± 82*	135 ± 116*	<380
(mg)	(23·0 ± 11·2)	(23·7 ± 10·8)	(17·7 ± 15·2)	< 50
Creatinine clearance ml/min	76 ± 34	78 ± 36	68 ± 21	> 80

Results asterisked were from a total of only 21 patients, 18 being in the cholestatic group and three in the hepatocellular group. i-PTH immunoreactive parathyroid hormone. 25-OHD: 25-hydroxy vitamin D.

one) plasma calcium values were less than 2·13 mmol/l (8·5 mg/100 ml) but, after correction for albumin levels, all values except in two PBC patients became normal. Low plasma phosphorus concentration stabilised to a low-normal range on a hospital diet. Only one patient (alcoholic hepatitis) had a greatly lowered plasma magnesium value of 0·33 mmol/l (0·8 mg/100 ml). Plasma alkaline phosphatase activity was increased in both groups and was significantly higher in the cholestatic when compared with the hepatocellular group ($P < 0·005$). All the serum i-PTH and plasma hydroxyproline results were within the normal range. Serum i-PTH did not correlate with any of the other measured biochemical variables. Serum 25-OHD values were low in hepatocellular disease and in all 32 patients correlated with plasma calcium (r 0·40, $P < 0·05$) but not with any of the other variables.

HISTOLOGICAL FINDINGS

Biopsies consisted of orderly lamellar bone. The iliac cortex varied from 0·1 mm to 3·0 mm in thickness. Cortices thinner than 1 mm were usual with osteoporotic biopsies. In two patients there was marked contrast between thick compact cortex and thin sparse underlying trabeculae.

Normal bone was found in PBC, (two); biliary atresia, (one); alcoholic hepatitis, (one); and cryptogenic cirrhosis (one).

Twenty-two patients (PBC, 15; secondary biliary cirrhosis, one; postoperative biliary stricture, one; sclerosing cholangitis, one; alcoholic cirrhosis, two, and alcoholic hepatitis, two) showed a slight to moderate osteomalacia (0·5% volume proportion of osteoid or more) with an excess of osteoid with values up to 5% Vv%. Both surface extent and maximal thickness increased with increasing osteoid volume.

Twelve of the patients (PBC, nine; secondary biliary cirrhosis, one; and alcoholic cirrhosis, two) showed volume proportion of total trabecular bone of less than 20% and were therefore defined as osteoporotic. In osteoporotic sections trabeculae were seen as a series of short narrow disconnected profiles.

Using a multiple regression of increase in osteoid against total bone, plasma calcium, and phosphorus, there was a significant correlation between osteoid volume and total bone volume ($P < 0·05$). All biopsies with an osteoid volume of greater than 1·5% showed a total bone of greater than 20% Vv. (Figure). Osteomalacia was therefore minimal in the presence of osteoporosis.

There was no evidence of increased osteoblasts on mineralised or osteoid covered surfaces. An occasional small osteoclast was seen in shallow excavations (one lamella deep) but tunnelling resorption was not observed. Histological evidence of excess parathyroid activity on bone (osteitis fibrosa) was entirely lacking.

EFFECT OF VITAMIN-D TREATMENT (Table 3)

The patients treated with regular vitamin D_2 had been jaundiced significantly longer than the untreated patients ($P < 0·05$) and also had had more prolonged liver symptoms ($P < 0·10$). The serum 25-OHD was higher in the treated patients ($P < 0·02$)

Table 3 *Biochemical results (mean ± SD) in group of patients with cholestatic liver disease when subdivided into those who had or had not received regular parenteral vitamin D_2 therapy*

	Treated	*Untreated*
Number of patients	16	9
Duration of symptoms (yr)	6·2 ± 2·7	4·0 ± 5·6
Years serum total bilirubin more than 51 μmol/l (3 mg/dl)	1·8 ± 1·6	0·7 ± 0·7
Bone pain or fractures	13	4
Plasma:		
calcium mmol/l (mg/dl)	2·30 ± 0·18 (9·2 ± 0·7)	2·22 ± 0·18 (8·9 ± 0·7)
corrected calcium mmol/l (mg/dl)	2·38 ± 0·20 (9·5 ± 0·8)	2·35 ± 0·18 (9·4 ± 0·7)
phosphorus mmol/l (mg/dl)	1·00 ± 0·13 (3·1 ± 0·4)	1·00 ± 0·19 (3·1 ± 0·6)
magnesium mmol/l (mg/dl)	0·88 ± 0·08 (2·14 ± 0·20)	0·90 ± 0·08 (2·20 ± 0·19)
total protein g/l	78 ± 11	81 ± 10
albumin g/l	37 ± 8	36 ± 8
alkaline phosphatase K.A. units/dl	53 ± 39	71 ± 28
Serum:		
i-PTH	0·31 ± 0·6	0·33 ± 0·9
25-OHD	18·1 ± 13·1	5·9 ± 4·6
24 h urinary		
calcium mmol (mg)	2·75 ± 1·2 (100 ± 48)	1·85 ± 1·65 (74 ± 59)
Bone osteoid (Vv%)	1·7 ± 1·6	1·1 ± 1·4
total bone (vv%)	22·4 ± 6·1	22·3 ± 9·0

i-PTH: immunoreactive parathyroid hormone. 25-OHD: 25-hydroxy vitamin D.

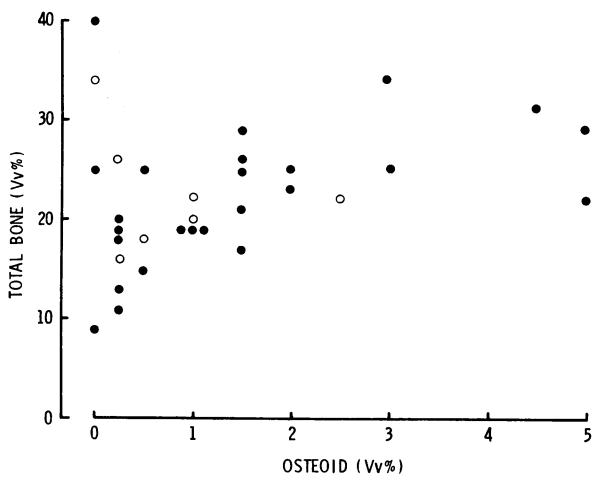

Figure *Graph of total bone (Vv%) and osteoid tissue (Vv%) in 32 patients. Values of less than 20% Vv total bone indicate osteoporosis and 0·5% Vv or more osteoid indicate osteomalacia. ○: hepatocellular disease; ●: cholestatic liver disease. Multiple regression with calcium and phosphorus,* r *0·40,* P < *0·05.*

and this was associated with higher 24 hour urinary calcium values (P < 0·10) and plasma corrected calcium values (P < 0·30). Despite these biochemical changes, increased osteoid tissue was if anything more marked in the treated patients.

CORRELATIONS BETWEEN HISTOLOGICAL, CLINICAL, AND BIOCHEMICAL DATA

There was no statistically significant correlation between any of the clinical data and the observed osteoid tissue or total bone values. Of the 22 patients with osteomalacia, 12 had bone pain and five of these had had recent fractures; nine of the 12 osteoporotic patients had bone pain and four of these had had recent fractures. The amount of osteoid tissue tended to increase with the number of years that the total bilirubin had been more than 51 μmol/l (3 mg/100 ml) (*r* 0·23) but less so with the duration of liver symptoms (*r* 0·04); these correlations were less for total bone.

Eleven of the 12 osteoporotic patients were over 45 years of age. Symptomatic osteoporosis was found in all three PBC patients who had received corticosteroid therapy; one of these had 1% volume increase in osteoid. Of the 10 patients (PBC, nine; biliary atresia, one) on cholestyramine, two had normal bone, six had varying degrees of osteomalacia, one had osteoporosis, and one had slight osteomalacia with osteoporosis. All the patients (PBC, three) receiving frusemide had osteomalacia; one also had osteoporosis. The patient (PBC) receiving phenobarbitone had osteomalacia.

There were no significant correlations between the plasma, serum, and urinary biochemical findings and the osteoid tissue or total bone values. There was a poor correlation (*r* − 0·10) between serum 25-OHD and increases in osteoid tissue. In particular, increased osteoid tissue did not correlate with an increased percentage of bone alkaline phosphatase isoenzyme; the increased total alkaline phosphatase

activity in these patients was mainly caused by increased liver isoenzyme. Serum 25-OHD was 15·1 ± 9·7 ng/ml in eight patients (PBC, six; secondary biliary cirrhosis, one; and biliary atresia, one) treated with vitamin D and cholestyramine and was not significantly different from the value of 21·2 ± 15·9 ng/ml in eight patients (PBC, eight) treated with vitamin D and no cholestyramine.

Discussion

A high incidence of both osteomalacia and osteoporosis was found with chronic cholestatic and with alcoholic liver disease. We are unaware of previous reports of osteomalacia in alcoholic liver disease. Symptomatic bone disease seems rare in non-alcoholic chronic hepatocellular disease (Paterson and Losowsky, 1967). The morbidity associated with both histological bone lesions was shown by the high incidence of bone pain and fractures. Osteomalacia was minimal when osteoporosis was present but the nature of the bony lesion could not be predicted by clinical and biochemical techniques. Radiology was also unreliable. The treatment for osteoporosis and osteomalacia is different and a bone biopsy is essential for accurate diagnosis.

The osteomalacia and osteoporosis were often associated with normal plasma and urinary biochemistry, especially after plasma calcium had been corrected for plasma albumin levels. However, vitamin D (Thompson *et al.*, 1966) and calcium absorption are abnormal in biliary disease and protein synthesis is reduced in cirrhosis. Plasma values do not necessarily reflect the whole body or bone status. Estimations of whole body and bone calcium, phosphorus, magnesium, and vitamin D could be helpful.

Although some of the biochemical findings, particularly serum 25-OHD, improved after parenteral vitamin D_2 therapy, bone disease was neither prevented nor cured. These observations are of importance in the aetiology of hepatic osteodystrophy. In the patients treated with vitamin D_2 the predominant circulating form of serum 25-OHD is probably vitamin D_2 rather than D_3. The findings in the patients studied here are of considerable interest when compared with the studies of Wagonfeld *et al.* (1976) who showed improvement or stabilisation of bone mineral content with oral 25-hydroxy vitamin D_3. Although vitamin D_2 is said to be as effective as D_3 in treating osteomalacia in man (Omdahl and DeLuca, 1973), this may not be so. Other possible reasons for therapeutic resistance include a failure of synthesis of dihydroxy vitamin D metabolites and end-organ unresponsiveness.

In the presence of osteomalacia, hypophosphataemia and creatinine clearance values of less than 70 ml/min in 13 patients, one would have expected raised levels of i-PTH. Radiological osteitis fibrosa has not been reported in chronic liver disease and was not seen in this series. The normal serum i-PTH and plasma and urinary hydroxy proline support normal parathyroid function; moreover, bone histology did not show the changes of hyperparathyroidism.

Diet and drugs may well be important in the development of metabolic bone disease. Alcoholics usually have a poor diet (Leevy *et al.*, 1970). Ethanol can cause osteoporosis and also has a direct effect on the gut inhibiting calcium absorption (Krawitt, 1975). Poor nutrition, including a lack of vitamin C, is a feature of other liver disease (Beattie and Sherlock, 1976). Low salts diets often contain a low daily calcium allowance. Cholestyramine has been shown to cause vitamin D malabsorption in rats (Thompson and Thompson, 1969) and relate to osteomalacia in man (Heaton *et al.*, 1972; Compston and Thompson, 1977). Frusemide and the thiazide diuretics increase urinary calcium excretion: in osteomalacia spironolactone may be superior. Phenobarbitone, now used as a choleretic, may cause osteomalacia by hepatic enzyme induction (Bowden, 1974). This study shows the osteoporotic effect of corticosteroids in PBC and as they have not been shown to be of therapeutic value their use appears to be contraindicated.

No single aetiological factor has emerged from this study to explain the osteomalacia and osteoporosis of liver disease. As in renal osteodystrophy the aetiology is likely to be multifactorial (Wills, 1971). In osteomalacia adequate parenteral vitamin D and oral calcium phosphorus and magnesium should be given to correct blood values to normal and, if possible, frusemide, cholestyramine, alcohol, and phenobarbitone should be avoided. In osteoporosis a diet containing high vitamin C and protein with the avoidance of corticosteroids and alcohol is recommended; mobilisation should be encouraged. Clinical trials of these measures together with bone biopsy data are needed.

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