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Clinical Investigation

Does "Hepatic Osteodystrophy" Differ from Peri- and Postmenopausal Osteoporosis? A Histomorphometric Study

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Summary. Bone remodeling activities were assayed on undecalcified sections of iliac crest biopsies obtained from patients with primary biliary cirrhosis (PBC) (n = 25), postmenopausal osteoporosis (n = 64), and age, sex-matched, nonosteoporotic peri- and postmenopausal subjects (n = 26). Thorough semiautomated static and dynamic bone histomorphometry was carried out. Statistical analysis was performed among the age-matched groups in 10-year intervals through the ages 45 to 74. No osteomalacia or osteoporotic condition was detected in the patients with PBC. Our data have shown they did not have a mineralization defect but rather high bone turnover rates. The discrepancy of our observations to other published studies is discussed. We suggest that the osteoporotic condition attributed by others to PBC may just be the result of the concomitant aging processes.

Key words: Primary biliary cirrhosis — Menopausal osteoporosis — Osteomalacia — Trabecular bone histomorphometry.

Specialists in the field of gastroenterology and metabolic bone diseases [1–3, 8] have drawn attention to the hepatic osteodystrophy resulting from primary biliary cirrhosis (PBC). A deficiency of Vitamin D metabolites has been incriminated for the development of osteomalacia in patients with PBC [5]. In addition, Atkinson et al. [2] postulated that defective cleavage of PTH by Kupffer's cells was responsible for the osteoporotic condition in patients with PBC. Therefore, patients with PBC were considered to have osteomalacia, or osteoporosis, or both [1, 4]. However, the varied techniques and insufficient information reported in the past resulted in some uncertainty of the interpretation of the histological changes of bone remodeling seen in patients with PBC. Osteomalacia should be defined histologically as an increase in both surface extent and width of osteoid seams accompanied by a mineralization defect. Osteoporosis should be regarded histologically as a condition of bone with an increase in cortical porosity, a decrease in total bone volume, and a thinning of both cortical and trabecular bone. An age, sex-matched analysis of bone of patients with PBC vs. controls was reported in only one article [8]. This analysis neither related to nor considered bone in menopausal osteoporosis. Due to the ambiguity of the hitherto reported histomorphometric data from bone of patients with PBC, we decided to effect a comparative statistical analysis between data from our records of the past 10 years. The present study reports the quantitative histomorphometric bone changes seen in female patients with PBC and patients at peri- and postmenopausal ages.

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Materials and Methods

Static and dynamic histomorphometric data are routinely evaluated on trabecular bone of undecalcified sections of iliac crest biopsies by a semiautomatic image analyzer (Kontron 64, Carl Zeiss Company, Toronto) using the "Osteoplan" software [9] in this metabolic bone disease laboratory. All data are classified according to the clinical and/or pathological diagnosis and stored in the data base. The data of the first biopsy from all women aged 45–74 classified either under "Primary Biliary Cirrhosis" (PBC) or under "Osteoporosis" were recalled. Data from 115 patients were obtained and categorized (Table 1) as follows: (1) data from subjects with PBC (all patients in our PBC files were

Table 1. Data from trabecular bone biopsy on 115 patients

Age	No. of data sets from subjects with PBC	No. with Normal volume	No. with Osteoporosis	Total
45–54	7	9	6	22
55–64	12	17	11	40
65–74	6	–	47	53
Total	25	26	64	115

women, had clinical and biochemical features of the disease, and had a diagnostic needle biopsy of liver); (2) data from subjects suspected of having osteoporosis but having a normal trabecular bone volume (in this category, a diagnosis of osteoporosis has been entertained due to chronic long-standing back pain but no radiological features of osteoporosis were apparent). In the 65–74 age group there were no "normal" controls in our female population; (3) data from subjects with osteoporosis whose trabecular bone volume was less than 15% per microscope field (in this category our women had clinical and radiological features of the disease). Laboratory tests ruled out any other etiology for the disease. Three 10-year intervals were arbitrarily selected in each category to minimize the interferences of age and menopause. Therefore, eight groups of data were accumulated (Table 1).

Histomorphometric Parameters

The following parameters were measured (160 ×; 16 × objective): (1) total bone volume, volumetric density of bone (TBV, mm^3/cm^3); (2) mean trabecular diameter (D-TRAB, μm); (3) volumetric density of lamellar osteoid (VV-OS 1, mm^3/cm^3); (4) relative volumetric density of lamellar osteoid (VV-O-1, mm^3/cm^3); (5) mean width of lamellar osteoid seams (TH-OS-1, μm); (6) fraction of trabecular surface covered by lamellar osteoid seams (OS-1, %); (7) fraction of osteoid covered by osteoblasts (OB/OS, %); (8) osteoblast index, number of osteoblasts per 10 cm of boundary length (OBI, n/10 cm); (9) fraction of trabecular surface with active and inactive resorptive lacunae (L-TOT, %); (10) fraction of active resorptive surface (OCL, %); (11) osteoclastic index (OCI, n/10 cm); (12) mean distance between double labels (MD-d, μm); (13) fraction of osteoid seams exhibiting double labels (LAB-os-d, %); (14) fraction of osteoid seams exhibiting single labels (LAB-os-s, %); (15) mean wall thickness of osteon (MWTH, μm); (16) corrected appositional rate (AR/day, μm/day); (17) bone formation rate, BMU level—surface referent (BFR-bmu, mm^3/mm^2/year); (18) mineralization lag-time (MLT, days); (19) osteon formation time (Sigma f, days); (20) osteon remodeling time (Sigma, days.)

Statistic Analysis

Unpaired Student *t* tests were carried out to compare the static and dynamic histomorphometric data between groups A and B and groups A and C under age-matched condition. The analysis was done by the IBM computer through the "Addstat" software program (Abacus Scientific Software).

Results

The comparisons of normal and PBC patients at ages 45–54 (Table 2) showed that thicker osteoid seams ($P < 0.05$), larger distances between double labels ($P < 0.02$), greater fraction of double-labeled surface ($P < 0.05$), faster appositional rate ($P < 0.02$), bone formation rate ($P < 0.02$), and thicker mean osteons ($P < 0.01$) in the PBC group. When compared, there was greater total trabecular bone volume ($P < 0.02$), thicker trabeculae ($P < 0.05$), thicker osteoid seams ($P < 0.05$), a smaller fraction of resorptive surface ($P < 0.05$), greater distances between double labels ($P < 0.01$), and faster appositional rate ($P < 0.05$) in the PBC group than in the osteoporotic group at ages 45–54 (Table 2).

Comparisons of the PBC and the normal group at ages 55–64 (Table 3) showed that mean distance between double labels ($P < 0.05$) and mean osteon thickness ($P < 0.01$) were significantly greater in the PBC group. There were greater total trabecular bone volumes ($P < 0.01$), thicker trabeculae ($P < 0.01$), thicker osteoid seams ($P < 0.02$), larger fractions of osteoblastic surfaces ($P < 0.01$), a higher number of osteoblasts per 10 cm bone surface ($P < 0.05$), greater distances between double labels ($P < 0.01$), and thicker osteons ($P < 0.01$) in the PBC group than in the osteoporotic group at ages 55–64 (Table 3).

The comparisons of the PBC group and the osteoporotic group at ages 65–74 showed that the dynamic remodeling activities parameters were significantly greater in the PBC group (Table 4).

Discussion

In the present study we analyzed the quantitative histomorphometric data routinely evaluated by semiautomatic image analyzer and stored in the data bank in this metabolic bone disease laboratory. The purpose was to find out if there were any significant variations of bone remodeling among age-matched female patients with postmenopausal osteoporosis, with primary biliary cirrhosis, and normal nonosteoporotic postmenopausal subjects. The results of bone remodeling activity measurements in our PBC patients is peculiar and contradictory to observations reported in other studies [3, 4, 8].

In the present investigation, the trabecular bone of the iliac crest in PBC patients shows similar volumes and diameters within age ranges of 45–64 (Table 2, 3). In addition, these measurements are significantly greater than those of the age-matched postmenopausal osteoporotic patients. This is indicative of normovolemic and nonosteoporotic

Table 2. PBC vs. normal and PBC vs. ORP—age 45–54

	Trabecular bone histomorphometry		
	Normal (n = 7)	PBC (n = 9)	ORP (n = 6)
V.V.	20.51 ± 4.01	22.08 ± 7.93	13.00 ± 1.84[e]
D-TRAB	304.43 ± 122.52	421.18 ± 143.08	250.06 ± 69.78[f]
V.V.-OS-1	0.39 ± 0.18	0.70 ± 0.40	0.33 ± 0.23
V.V.-O-1	1.83 ± 1.22	2.97 ± 0.82	2.70 ± 2.06
TH-OS-1	8.88 ± 2.68	14.03 ± 5.87[a]	7.31 ± 1.98[f]
OS-1-%	14.40 ± 9.52	19.46 ± 5.86	17.53 ± 11.18
OB/OS-%	9.33 ± 8.36	14.50 ± 7.59	6.07 ± 5.98
OB1	126.86 ± 151.07	146.79 ± 71.31	130.52 ± 196.49
L-TOT-%	4.62 ± 2.97	3.06 ± 1.70	5.74 ± 2.40[f]
OCL%	2.32 ± 1.92	1.94 ± 0.93	2.18 ± 2.33
OCI	46.76 ± 73.82	45.61 ± 29.29	44.07 ± 46.51
MD-d	12.44 ± 5.34	21.30 ± 6.40[b]	8.83 ± 1.88[d]
LAB-OS-d	34.00 ± 19.52	61.51 ± 21.45[c]	58.4 ± 42.34
LAB-OS-s	28.56 ± 14.27	102.34 ± 121.59	81.08 ± 63.51
MWTH	39.19 ± 9.40	66.75 ± 25.82[a]	42.50 ± 8.05
AR/D	0.29 ± 0.18	0.64 ± 0.26[b]	0.31 ± 0.22[f]
BRF bmu	0.06 ± 0.05	0.21 ± 0.13[b]	0.10 ± 0.15
MIN-LAG-T	50.16 ± 33.29	24.04 ± 10.58	34.89 ± 24.26
SIGMA f	222.94 ± 206.08	127.33 ± 88.16	186.10 ± 97.59
SIGMA	361.84 ± 389.62	149.24 ± 87.81	261.42 ± 137.40

Data expressed as mean ± SD

[a,b,c] Statistical significance at the level $P < 0.01$, $P < 0.02$, $P < 0.05$, respectively in the comparisons of PBC vs. normal

[d,e,f] Statistical significance at the level $P < 0.01$, $P < 0.02$, $P < 0.05$, respectively in comparisons of PBC vs. ORP

conditions in the PBC patients. Although static parameters do show significantly thicker osteoid seams in PBC patients of the above-mentioned age range than those of the matched control subjects and osteoporotic patients, there is evidence that dynamic parameters are also more significantly altered in the PBC patients. There are indications of a sound mineralization rate of the osteoid at this skeletal site sampled in the PBC patients. Therefore, the PBC patients evaluated by this laboratory have neither an osteomalacic nor an osteoporotic condition. On the contrary, they demonstrate a form of bone remodeling that is faster than that of the age, sex-matched, normal nonosteoporotic postmenopausal subjects.

However, these features change slightly when PBC patients are between 65 and 74 years. The trabecular bone volume and diameter as well as other static parameters are not significantly different from those of age-matched osteoporotic patients, yet, the dynamic parameters indicate that the PBC patients are devoid of a mineralization defect, as is shown in the age-matched osteoporotic patients (Table 4). The decline of trabecular bone volume in iliac crest bone biopsies from patients with PBC is remarkable at ages 65–74 as compared to that of the other two age ranges. This may result from the slightly increased bone resorption activity and the prolonged mineralization lag-time in these patients under the influence of the aging processes.

Histomorphometric studies published in the past show a large discrepancy in interpretation of the mechanisms for osteodystrophy in PBC. But generally, an osteomalacic condition is not observed [3, 4, 8] and osteoporosis is incriminated for the fractures and bone pain among the patients with PBC [4, 6]. However, the age of the patients in some of the studies has covered a large range, from early 20s to late 60s. Among these studies, only one has age-matched comparisons using 10-year intervals in female patients [8], and two studies have sex-matched comparisons [4, 8]. Although different histomorphometric technologies were used in these studies, the data obtained from normal subjects of the first two age groups in the present study are closely matched to the control data of others.

Cuthbert et al. [3] stated that although there was no evidence of osteomalacia and osteoporosis on initial bone histomorphometry, the increased resorption surface might be indicative of a faster bone turnover. The small number of patients and wide range of age among their patients may account for the discrepancy with our series of patients. A decreased bone (osteoid) formation and a mineralization defect are features of the osteoporosis observed by Hodgson et al. [4] and Stellon et al. [8]. In fact, the total trabecular bone volume in the PBC patients in these studies was not significantly different from the normal control (also, the labeling escape phenomenon was not considered in these

Table 3. PBC vs. normal—age: 55–64

	Trabecular bone histomorphometry		
	Normal (n = 12)	PBC (n = 17)	ORP (n = 11)
V.V.	19.32 ± 3.43	21.26 ± 4.51	11.80 ± 2.77[c]
D-TRAB	347.51 ± 94.80	412.74 ± 87.28	278.02 ± 86.98[c]
V.V.-OS-1	0.49 ± 0.38	0.93 ± 1.19	0.31 ± 0.19
V.V.-O-1	2.51 ± 1.95	4.21 ± 5.06	2.55 ± 1.26
TH-OS-1	9.50 ± 3.04	13.24 ± 7.12	7.72 ± 2.05[d]
OS-1-%	18.86 ± 9.67	25.11 ± 21.77	19.44 ± 7.06
OB/OS-%	10.80 ± 11.86	10.24 ± 5.53	4.39 ± 3.18[c]
OB1	116.88 ± 88.28	156.22 ± 123.89	61.80 ± 59.45[e]
L-TOT-%	3.60 ± 1.96	2.97 ± 2.38	4.60 ± 3.05
OCL %	1.52 ± 1.19	1.88 ± 1.07	1.98 ± 1.66
OCI	31.03 ± 31.93	31.17 ± 18.70	43.04 ± 42.26
MD-d	11.93 ± 3.76	16.39 ± 5.46[b]	10.54 ± 3.27[c]
LAB-OS-d	60.13 ± 39.26	55.86 ± 28.98	40.47 ± 28.76
LAB-OS-s	72.98 ± 41.28	43.08 ± 39.15	66.85 ± 42.30
MWTH	36.34 ± 12.23	54.67 ± 20.72[a]	34.09 ± 8.24[c]
AR/D	0.57 ± 0.35	0.51 ± 0.34	0.28 ± 0.18
BFR bmu	0.24 ± 0.19	0.18 ± 0.17	0.07 ± 0.08
MIN-LAG-T	64.84 ± 165.05	38.50 ± 32.12	57.76 ± 63.51
SIGMA f	171.44 ± 317.52	216.40 ± 292.76	314.74 ± 516.08
SIGMA	211.64 ± 390.00	263.57 ± 325.80	484.01 ± 946.98

Data expressed as mean ± SD
[a,b] Statistical significance at the level $P < 0.01$, $P < 0.05$, respectively in the comparisons of PBC vs. normal
[c,d,e] Statistical significance at the level $P < 0.01$, $P < 0.02$, $P < 0.05$, respectively in comparisons of PBC vs. ORP

Table 4. PBC vs. ORP—age: 65–74

	Trabecular bone histomorphometry	
	PBC (n = 6)	ORP (n = 47)
V.V.	13.47 ± 4.05	14.19 ± 4.28
D-TRAB	379.16 ± 105.91	334.06 ± 116.51
V.V.-OS-1	0.27 ± 0.19	0.32 ± 0.29
V.V.-O-1	2.03 ± 1.14	2.26 ± 1.87
TH-OS-1	11.14 ± 4.37	8.54 ± 3.56
OS-1-%	16.48 ± 10.33	19.05 ± 12.15
OB/OS-%	15.02 ± 13.79	10.09 ± 8.89
OB1	174.90 ± 185.54	149.40 ± 183.43
L-TOT-%	3.00 ± 1.29	4.69 ± 3.18
OCL %	2.84 ± 1.79	2.35 ± 2.47
OCI	49.19 ± 34.91	35.12 ± 35.01
MD-d	15.92 ± 5.20	10.62 ± 4.14
LAB-OS-d	99.82 ± 91.85	44.00 ± 28.40
LAB-OS-s	122.13 ± 115.50	68.13 ± 51.78
MWTH	54.44 ± 11.14	43.00 ± 16.14
AR/D	0.82 ± 0.72	0.33 ± 0.28
BFR bmu	0.63 ± 0.78	0.10 ± 0.15
MIN-LAG-T	65.80 ± 105.66	44.55 ± 34.35
SIGMA f	539.49 ± 1103.02	247.13 ± 225.36
SIGMA	613.9 ± 1215.52	358.24 ± 317.10

Data expressed as mean ± SD
[a,b] Statistical significance at the level $P < 0.01$, $P < 0.05$, respectively in comparisons of PBC vs. ORP

studies). In the study done by Hodgson et al. [4] because of lack of double labeling in many patients, statistical analysis was carried out differently than in our study.

Because PBC often occurs in the middle-aged woman, the menopausal status is important. However, hyperestrogenemia is reported in the PBC patients [10]. Administration of estrogen can increase bone mineral content but does not increase total bone mass (Grynpas and Anderson, unpublished data, 1987). Our data suggest that the menopause might not affect PBC patients and that the osteoporotic condition seen between ages 65 and 74 may have resulted from involutionary processes.

The present investigation does not compare the data on serum biochemistry in patients with PBC. Nevertheless, the serum calcium, phosphate, and iPTH levels are reported to be within normal ranges or unchanged in patients treated with vitamin D in other studies [3, 4, 8]. Although Vitamin D supplement is recommended for PBC patients [12], its effectiveness is doubted by other studies [1, 5, 6, 8].

The tendency of our PBC patients to have thicker trabeculae and osteons suggests that the balance of bone remodeling is on the positive side. The significantly thicker osteoid and wider distance between double labels in PBC patients indicates a high turnover rate of bone remodeling, although the activation frequency judged from the fractions of osteoid surfaces and active resorbing surfaces is not significantly greater than the age-matched normal subjects. This is in agreement with the statements postulated by other studies [1, 3] that a "high turnover" of bone remodeling may have occurred in PBC patients.

At the present time, this laboratory has not accumulated enough of these PBC patients (with second iliac crest biopsy 2 years after the first biopsy in each age range) to examine whether osteoporosis occurs in these peri- and postmenopausal patients with PBC, although preliminary observations appear to indicate that they do not seem to be affected by the menopause.

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References

1. Arnaud SB (1982) 25-hydroxyvitamin D treatment of bone disease in primary biliary cirrhosis. Gastroenterology 83:137–149
2. Atkinson MJ, Vido I, Keck E, Hesch R-D (1983) Hepatic osteodystrophy in primary biliary cirrhosis: a possible defect in Kupffer cell mediated cleavage of parathyroid hormone. Clin Endocrinol 18:21–28
3. Cuthbert JA, Pak CYC, Zerwekh JE, Glass KD, Combes B (1984) Bone disease in primary biliary cirrhosis: increased bone resorption and turnover in the absence of osteoporosis or osteomalacia. Hepatology 4:1–8
4. Hodgson SF, Dickson ER, Wahner HW, Johnson KA, Mann KG, Riggs BL (1985) Bone loss and reduced osteoblast function in primary biliary cirrhosis. Ann Int Med 103:855–860
5. Long RG, Wills MR (1978) Hepatic osteodystrophy. Br J Hosp Med ii:312–321
6. Matloff DS, Kaplan MM, Neer RM, Goldberg MJ, Bitman W, Wolfe HJ (1982) Osteoporosis in primary biliary cirrhosis: effects of 25-hydroxyvitamin D_3 treatment. Gastroenterology 83:97–102
7. Mawer EB, Klass HJ, Warnes TW, Berry JL (1985) Metabolism of vitamin D in patients with primary biliary cirrhosis and alcoholic liver disease. Clin Sci 69:561–570
8. Stellon AJ, Webb A, Compston J, Wilbrams R (1986) Lack of osteomalacia in chronic cholestatic liver disease. Bone 7:181–185
9. Malluche HH, Sherman D, Meyer W, Massry SG (1982) A new semiautomatic method for quantitative static and dynamic bone histology. Calcif Tissue Int 34:439–448
10. Pentikainen PJ, Pentikainen LA, Azarnoff DL (1975) Plasma levels and excretion of estrogens in urine in chronic liver disease. Gastroenterology 69:20–27
11. Weaver GA, Franck WA, Streck WF, Starzl TE (1983) Hepatic osteodystrophy after liver transplantation in a patient with primary biliary cirrhosis. Am J Gastroenterol 78:102–106
12. Rao DS, Villaneuva A, Mathew M (1983) Histologic evolution of vitamin D depletion in patients with intestinal malabsorption or dietary deficiency. In: Fram B, Potts JT (eds) Clinical disorders of bone and mineral metabolism. Excerpta Media, Amsterdam, pp. 225–226

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