Vitamin D and Parathyroid Hormone in Outpatients With Noncholestatic Chronic Liver Disease

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Background & Aims: **The liver plays a central role in vitamin D metabolism. Our aim was to determine the prevalence and type of vitamin D–parathyroid hormone (PTH) disturbance in ambulatory patients with noncholestatic chronic liver disease (CLD) and its relationship with disease severity and liver function.** ***Methods:*** **We studied 100 consecutive outpatients (63 men, 37 women; mean age, 49.0 ± 12.1 [SD] y) with noncholestatic CLD caused by alcohol (n = 40), hepatitis C (n = 38), hepatitis B (n = 12), autoimmune hepatitis (n = 4), hemochromatosis (n = 4), and nonalcoholic steatohepatitis (n = 2); 51 patients had cirrhosis. Serum concentrations of 25-hydroxyvitamin D (25[OH]D), PTH, calcium, phosphate, magnesium, creatinine, and liver function tests were determined.** ***Results:*** **Serum 25(OH)D levels were inadequate in 91 patients: vitamin D deficiency (<50 nmol/L) was found in 68 patients and vitamin D insufficiency (50–80 nmol/L) was found in 23 patients. Secondary hyperparathyroidism (serum PTH, >6.8 pmol/L) was present in 16 patients. The prevalence of vitamin D deficiency was significantly higher in cirrhotic vs noncirrhotic patients (86.3% vs 49.0%; *P* = .0001). In Child–Pugh class C patients, 25(OH)D levels were significantly lower than in class A patients (22.7 ± 10.0 nmol/L vs 45.8 ± 16.8 nmol/L; *P* < .001). Serum 25(OH)D independently correlated with international normalized ratio (negatively; *P* = .018) and serum albumin (positively; *P* = .007). Serum 25(OH)D levels of less than 25 nmol/L predicted coagulopathy, hyperbilirubinemia, hypoalbuminemia, increased alkaline phosphatase, and anemia and thrombocytopenia.** ***Conclusions:*** **Vitamin D inadequacy is common in noncholestatic CLD and correlates with disease severity, but secondary hyperparathyroidism is relatively infrequent. Management of CLD should include assessment of vitamin D status in all patients and replacement when necessary.**

The liver is a major organ in the vitamin D endocrine system. To function physiologically vitamin D must first be converted in the liver to 25-hydroxyvitamin D (25[OH]D), the main circulating form of vitamin D, and this in turn is converted in the kidney into 1, 25-dihydroxyvitamin D, the active metabolite.[1–3] Liver cells along with parathyroid glands and kidneys express a calcium-sensing receptor4 that plays a critical role in regulating systemic calcium homeostasis.

In recent years it has been recognized that the vitamin D endocrine system is not only the principal regulator of calcium and phosphate homeostasis and bone metabolism, but it also exerts potent noncalciotropic functions including antiproliferative, prodifferentiative, and immunomodulatory activities.[3] Vitamin D insufficiency has been linked, apart from osteoporosis, to a wide range of inflammatory, autoimmune, and metabolic disorders and malignancies.[5] On the other hand, the normal liver is a target organ for the vitamin D endocrine system[6] and parathyroid hormone (PTH).[7,8]

Although chronic liver disease (CLD), especially cholestatic, alcoholic, and in advanced stages from any causes, often (20%–60%) is complicated by bone disease,[9–11] the clinical relevance of vitamin D–PTH disturbances in hepatic osteodystrophy still is unclear.[9,12–22]

Vitamin D deficiency traditionally is considered to cause secondary hyperparathyroidism and this has been observed in up to 42% of patients with CLD in some studies,[16,22] whereas in other studies the PTH levels were normal[23] or even low.[24–26]

The few studies correlating 25(OH)D–PTH status and severity of the liver injury reported conflicting results. Some investigators[13,18,25–28] have suggested that 25(OH)D levels decrease with disease progression, but others did not find differences between cirrhotic and noncirrhotic patients[22] or between Child–Pugh groups.[24]

Despite accumulating evidence that vitamin D has a number of actions that may be relevant to liver function and CLD, including the regulation of secretion of metalloproteinases and their inhibitors, fibroblast proliferation, and collagen synthesis,[29,30] currently the evaluation and correction of vitamin D status is not part of the routine management of these patients.

It also should be noted that most work has been performed in patients with chronic cholestatic liver disease (particularly primary biliary cirrhosis) and studies often were limited by small numbers. In the present study we investigated vitamin D and PTH status in a diverse ambulant group of patients with noncholestatic CLD and a range of disease activity. Our aims were to determine the prevalence, extent, and type of disturbances in calcium–vitamin D–PTH status and its relationship with the severity of disease and liver function injury.

Materials and Methods

Patients

One hundred consecutive patients attending the outpatient clinic of the Gastroenterology Department at Canberra

Abbreviations used in this paper: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLD, chronic liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; GGT, γ-glutamyltransferase; INR, international normalized ratio; MMP, matrix metalloproteinase; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

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Hospital in whom there was a confirmed diagnosis of noncholestatic CLD were included in this study. The group consisted of 63 men and 37 women, with a mean age of 49.0 ± 12.1 (SD) years. The cause of CLD was alcohol use (n = 40), viral hepatitis C (n = 38), viral hepatitis B (n = 12), autoimmune hepatitis (n = 4), hemochromatosis (n = 4), and nonalcoholic steatohepatitis (n = 2). The diagnosis of CLD was based on consistent clinical findings, serologic markers (antibodies to hepatitis C virus [anti-HCV], hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, hepatitis B e antigen, hepatitis B e antibody, hepatitis B virus DNA, and HCV RNA measurements by polymerase chain reaction, autoantibodies [antinuclear antibody, anti–smooth muscle antibody]), biochemical features (including iron studies and hemochromatosis gene test), endoscopic (n = 50) and imaging (including ultrasound, n = 100) evidence, and histologic examinations (liver biopsy examination, n = 37). Of 38 patients with CLD as a result of HCV, 8 subjects (including 4 with cirrhosis) had a history of alcohol overuse; the HCV cause was confirmed by liver biopsy examination in 2 of these patients. None of the patients had steatorrhea. The severity of cirrhosis was graded using the Child–Pugh score and patients were grouped into 3 categories: class A (scores 5–6; n = 15), class B (scores 7–9; n = 16), or class C (scores 10–15; n = 20). The Model for End-Stage Liver Disease score also was calculated according to the United Network for Organ Sharing formula.[31] None of the patients received vitamin D or calcium supplements, bisphosphonates, calcitonin, or hormone replacement therapy. Twenty-one patients were treated with spironolactone, 14 with furosemide, 6 with lamivudine (one of whom was also on adefovir), 5 with combination peginterferon alfa-2a or 2b and ribavirin, and 4 were on corticosteroids. Seven other patients had previously received either standard or pegylated interferon alone or in combination with ribavirin for hepatitis C treatment.

All patients were residents of the Canberra region (latitude, 33° 15′ S). All patients gave their informed consent to participate in the study, which was approved by the local ethics review committee.

Laboratory Analysis

Samples of venous blood were obtained in the morning after an overnight fast and were kept frozen at −70°C for the assay of 25(OH)D and intact PTH. The tests were performed using commercially available kits according to the manufacturers' instructions. 25(OH)D was measured with ^{125}I radioimmunoassay kit (DiaSorin, Stillwater, MN); the intra-assay and interassay coefficient of variation were 8.6% and 9.4%, respectively. The laboratory reference range was 31–107 nmol/L. Intact PTH was measured by 2-site chemiluminescent enzyme-labeled immunoassay for the 1–84 amino acid chain on the Immunolite 2000 auto-analyzer (Diagnostics Products Corporation, Los Angeles, CA); intra-assay and interassay coefficients of variation were 5.2% and 6.3%, respectively. The laboratory reference range was 1.3–6.8 pmol/L.

All subjects had serum total calcium, phosphate, magnesium, albumin, total bilirubin, aminotransferases, alkaline phosphatase, prothrombin time, creatinine, urea, sodium, potassium, glucose, hemoglobin, and full blood count determined by routine laboratory techniques. The serum calcium level was corrected for albumin concentration; the international normalized ratio (INR) for prothrombin time was calculated.

On the basis of data reported in the literature,[32,33] the serum 25(OH)D concentration was defined as deficient when it was less than 50 nmol/L (severe deficiency, <12.5 nmol/L; moderate deficiency, 12.5–25 nmol/L; mild deficiency, 25–49 nmol/L), insufficient when it was 50–80 nmol/L, and sufficient (adequate, desirable, normal) when it exceeded 80 nmol/L.

Statistical Analysis

Statistical analyses were performed with a statistical software package (Stata version 7; Stata Press, College Station, TX). Data are presented as mean values and SDs. For differences between groups, significance was assessed using an unpaired 2-tailed Student *t* test for continuous variables and the Pearson χ^2 test with the Fisher exact test for categoric variables. Correlations between 25(OH)D, PTH, biochemical markers of liver function, and demographic parameters were examined using linear regression analysis and the Pearson correlation coefficient. Multiple linear regression analysis was performed to identify independent variables associated with a low 25(OH)D level. The appropriateness of the regression model was assessed by Jack-knife residuals, Cook's d, and Mallow's C_p. A *P* value of less than .05 was considered statistically significant.

Results

Patient Characteristics

The patients were diverse in age, nature, and severity of their noncholestatic CLD. Table 1 summarizes the demographic, etiologic, and main biochemical and hematologic data on all subjects studied. The age of the patients ranged from 22 to 76 years. The cirrhotic patients were significantly older than the noncirrhotic patients, but there was no difference between the 2 groups regarding sex, with a male predominance in both groups. The main causative factor for cirrhosis was alcohol (72.5%), whereas in the noncirrhotic group it was viral hepatitis C (57.1%) and B (22.4%). As expected, the biochemical parameters of liver function and hematologic characteristics differed significantly between the cirrhotic and noncirrhotic patients. The former group had higher mean INR values, increased concentrations of serum bilirubin, alkaline phosphatase (ALP), γ-glutamyl transferase, and lower serum albumin, alanine aminotransferase, hemoglobin, and platelets. These differences were not related to sex and were more pronounced in advanced stages of cirrhosis. No differences were seen between the groups for creatinine and urea concentrations.

Vitamin D Status

In total, inadequate vitamin D status, defined as a serum 25(OH)D level lower than 80 nmol/L, was present in 91 patients, vitamin D deficiency (<50 nmol/L) was seen in 68 patients, and insufficiency (50–80 nmol/L) was seen in 23 patients. Only 9 noncirrhotic patients showed a normal (>80 nmol/L) serum 25(OH)D concentration (Table 2). There was no difference in serum 25(OH)D levels between the sexes (42.6 ± 28.0 nmol/L in men vs 43.3 ± 21.9 nmol/L in women).

The severity of CLD and the stage of cirrhosis according to Child–Pugh classification and Model for End-Stage Liver Disease score showed significant correlation with the serum 25(OH)D concentration (Table 2). The mean serum concentration of 25(OH)D was significantly lower in patients with cirrhosis compared with noncirrhotic patients. When patients

Table 1. Demographic, Clinical, Hematologic, and Serum Biochemical Characteristics of Studied Patients With Noncholestatic CLD

Characteristics	Cirrhotic patients (n = 51)	Noncirrhotic patients (n = 49)	*P* value
Age, *y*			
Range	37–76	22–66	
Mean ± SD	55.0 ± 9.6	42.8 ± 11.2	<.001
Men, n (%)	36 (70.6%)	27 (55.1%)	.163
Disease cause			
Alcohol, n (%)	37 (72.5%)	3 (6.1%)	<.001
HCV, n (%)	10 (19.6%)	28 (57.1%)	<.001
HBV, n (%)	1 (2.0%)	11 (22.4%)	.005
Nonalcoholic steatohepatitis	2 (3.9%)	0	.493
Autoimmune	1 (2.0%)	3 (6.1%)	.581
Hemochromatosis	0	4 (8.2%)	.116
Total bilirubin level, *μmol/L*	59.7 ± 63.5	12.6 ± 12.6	<.001
Albumin level, *g/L*	31.8 ± 8.6	41.7 ± 3.4	<.001
ALT level, *U/L*	49.4 ± 38.9	122.8 ± 189.8	.010
ALP level, *U/L*	175.4 ± 118.9	93.0 ± 38.7	<.001
GGT level, *U/L*	231.8 ± 302.3	103.2 ± 113.6	.006
INR, *U/L*	1.5 ± 0.36	1.1 ± 0.24	<.001
Hemoglobin level, *g/L*	116.1 ± 24.7	146.6 ± 15.9	<.001
Platelet level, $\times 10^9$/L	151.5 ± 87.9	235.9 ± 50.0	<.001
Creatinine count, *μmol/L*	80.6 ± 28.1	75.4 ± 13.2	.236
Urea level, *mmol/L*	6.0 ± 6.1	4.9 ± 1.5	.218

HCV, hepatitis C virus; HBV, hepatitis B virus; ALT, alanine aminotransferase; GGT, γ-glutamyltransferease.

were classified according to their severity of liver disease there was a consistent trend for lower 25(OH)D levels with increasing severity of cirrhosis. None of the cirrhotic patients had a desirable level of serum 25(OH)D. The percentages of subjects with severe to moderate vitamin D deficiency (<25 nmol/L) were as follows: Child–Pugh class A, 6.7%; class B, 43.8%; and in class C, 65%. The mean 25(OH)D concentration in class C patients was 2 times lower than in class A patients ($P = .001$).

Calcium, Phosphate, and Magnesium

The mean concentrations of corrected calcium, inorganic phosphate, and magnesium showed no difference between cirrhotic and noncirrhotic patients, nor among cirrhotic subgroups. However, a low serum magnesium level (<.70 mmol/L) was found more often in subjects with cirrhosis than in noncirrhotic patients (16 of 51 vs 2 of 49; $P = .001$). Of 16

Table 2. Serum Concentrations of 25(OH)D, PTH, Calcium, Phosphate, and Magnesium in Patients With Cirrhosis Classified According to Child–Pugh Score and Noncirrhotic Patients With CLD

	Cirrhotic patients					
	Child–Pugh class			Total	Noncirrhotic patients	
	A (n = 15)	B (n = 16)	C (n = 20)	(n = 51)	(n = 49)	*P* value[a]
Age, *y*	53.4 ± 9.1	53.9 ± 10.0	57.1 ± 9.8	55.0 ± 9.6	42.8 ± 11.2	<.001
Men, n (%)	8 (53.3%)	12 (75.0%)	16 (80%)	36 (70.6%)	27 (55.1%)	.163
25(OH)D, *nmol/L*	45.8 ± 16.8	32.4 ± 20.1	22.7 ± 10.0	32.6 ± 18.2	53.6 ± 28.3	<.001
<25 *nmol/L*, n (%)	1 (6.7%)	7 (43.8%)	13 (65.0%)	21 (41.2%)	7 (14.3%)	.006
25–49 *nmol/L*, n (%)	9 (60.0%)	7 (43.8%)	7 (35%)	23 (45.1%)	17 (34.7%)	.005
50–80 *nmol/L*, n (%)	5 (33.3%)	2 (12.5%)	0	7 (13.7%)	16 (32.6%)	.010
>80 *nmol/L*, n (%)	0	0	0	0	9 (18.4%)	.005
Calcium level, *mmol/L*	2.33 ± 0.10	2.37 ± 0.12	2.37 ± 0.14	2.36 ± 0.12	2.35 ± 0.09	.639
Phosphate level, *mmol/L*	1.17 ± 0.20	1.13 ± 0.31	1.06 ± 0.28	1.11 ± 0.27	1.15 ± 0.20	.401
Magnesium level, *mmol/L*	0.76 ± 0.11	0.76 ± 0.11	0.77 ± 0.18	0.76 ± 0.14	0.84 ± 0.09	.085
PTH level, *pmol/L*	4.7 ± 2.3	3.8 ± 2.4	4.7 ± 3.0	4.4 ± 2.6	4.7 ± 2.7	.572
>6.8 *pmol/L*, n (%)	3 (20.0%)	3 (18.8%)	2 (10.0%)	8 (15.7%)	8 (16.3%)	1.000
<1.3 *pmol/L*, n (%)	0	2 (12.5%)	1 (5.0%)	3 (5.9%)	3 (6.1%)	1.000
Child–Pugh score	5.4 ± 0.51	8.0 ± 0.89	11.4 ± 1.35	8.6 ± 2.69		
MELD score[b]	9.0 ± 1.88	12.5 ± 3.80	19.8 ± 4.26	11.0 ± 5.61	7.58 ± 2.25	<.001

[a]Comparing cirrhotic and noncirrhotic patients.

[b]Model for End-Stage Liver Disease (MELD) score was calculated according to the United Network for Organ Sharing formula.[31]

Table 3. Demographic, Biochemical, and Hematologic Data in Patients With CLD by Serum 25(OH)D Concentration

	Serum 25(OH)D, *nmol/L*				
	<25 (n = 28)	25–49 (n = 40)	50–80 (n = 23)	>80 (n = 9)	*P* value[a]
Age, *y*	51.4 ± 11.6	48.6 ± 11.5	48.9 ± 13.8	43.7 ± 11.4	.412
Men, n (%)	19 (67.9%)	26 (65.0%)	12 (52.2%)	6 (66.7%)	.529
25(OH)D, *nmol/L*	16.9 ± 6.0	37.7 ± 6.5	60.6 ± 7.7	101.3 ± 22.7	<.001
INR	1.5 ± 0.4	1.3 ± 0.4	1.1 ± 0.1	1.0 ± 0.1	<.001
Bilirubin level, *μmol/L*	62.8 ± 63.1	37.0 ± 54.7	15.6 ± 11.9	7.3 ± 2.4	.002
Albumin level, *g/L*	29.4 ± 7.5	38.1 ± 7.9	41.0 ± 4.7	41.6 ± 3.0	<.001
ALT level, *U/L*	109.4 ± 240.6	68.2 ± 75.8	77.3 ± 57.4	107.7 ± 75.8	.637
ALP level, *U/L*	184.2 ± 105.4	126.3 ± 108.2	108.3 ± 61.4	89.0 ± 19.5	.094
GGT level, *U/L*	197.2 ± 178.9	196.6 ± 328.4	115.2 ± 119.8	94.0 ± 74.2	.396
Creatinine level, *μmol/L*	85.3 ± 30.6	76.6 ± 14.5	73.3 ± 23.6	74.2 ± 8.7	.211
Urea level, *mmol/L*	6.3 ± 7.9	5.1 ± 2.0	5.4 ± 1.9	4.7 ± 1.0	.714
Calcium corrected, *mmol/L*	2.35 ± .10	2.35 ± .12	2.37 ± .10	2.35 ± .09	.828
Phosphate level, *mmol/L*	1.11 ± .23	1.15 ± .29	1.15 ± .18	1.09 ± .18	.803
Magnesium level, *mmol/L*	.76 ± .15	.82 ± .13	.80 ± .08	.83 ± .04	.168
PTH level, *pmol/L*	4.8 ± 3.0	4.2 ± 2.7	4.6 ± 2.2	5.5 ± 2.2	.527
>6.8 *pmol/L*, n (%)	4 (14.3%)	5 (12.5%)	4 (17.4%)	3 (33.3%)	.451
Hemoglobin level, *g/L*	116.5 ± 24.8	132.3 ± 25.2	138.7 ± 24.5	150.9 ± 7.9	<.001
Platelet count, $\times 10^9$/L	166.0 ± 97.6	192.8 ± 80.6	207.0 ± 66.9	240.6 ± 61.5	.086

ALT, alanine aminotransferase; ALD, alkaline phosphatase; GGT, γ-glutamyltransferase; PTH, parathyroid hormone.
[a]Test for linear trend.

cirrhotic patients with hypomagnesaemia, 6 had renal impairment and 1 was receiving corticosteroids.

Parathyroid Hormone Status

There was no difference in serum PTH concentrations between cirrhotic and noncirrhotic patients, nor between the Child–Pugh groups (Table 2). The percentage of patients with increased PTH levels (>6.8 pmol/L) was equal in both groups (15.7% and 16.3% in cirrhotic and noncirrhotic patients, respectively). Three patients in each group had PTH levels lower than the lower level of reference interval (<1.3 pmol/L). Of 16 patients with increased PTH levels, 3 had mildly increased serum creatinine concentrations (>90 μmol/L, upper limit of reference range). Four of these 16 patients were taking spironolactone, 3 were taking furosemide, 1 was taking corticosteroids, and 1 was taking combination treatment with peginterferon alfa-2a and ribavirin. Of 6 patients with low PTH levels, 2 received spironolactone, 1 received furosemide, and 1 received corticosteroids. The chemistry test results showed no significant differences for albumin, calcium, phosphate, and magnesium among patients with increased or suppressed PTH levels compared with those with normal serum PTH concentrations. There were no differences in serum PTH levels between men and women.

Relationship Between Serum 25-Hydroxyvitamin D Level and Markers of Liver Disease, Parathyroid Hormone, Calcium, Phosphate, and Magnesium

Table 3 shows that when categorized by level of serum 25(OH)D, laboratory markers of CLD differed significantly and the severity of vitamin D inadequacy paralleled the changes. Patients with vitamin D deficiency (<50 nmol/L) had significantly higher values of INR, serum concentrations of bilirubin, ALP, and γ-glutamyl transferase, and lower levels of albumin, hemoglobin, and platelets compared with subjects with vitamin D insufficiency (50–80 nmol/L) or normal (>80 nmol/L) 25(OH)D levels. The mean difference between the groups of patients with lowest and highest serum 25(OH)D levels were as follows: for INR, 0.5; for albumin, 12.2 g/L; for bilirubin, 55.5 pmol/L; for ALP, 95.2 mmol/L; for γ-glutamyl transferase, 103.2 mmol/L; for hemoglobin, 34.4 g/L; and for platelets, 74.6 × 10 g/L. In contrast, the mean values of serum PTH, calcium corrected, and phosphate as well as age and sex showed no association with vitamin D status and no difference between groups with the lowest and highest 25(OH)D levels were seen.

The results of linear regression analysis relating serum 25(OH)D concentrations to laboratory indices of liver function, serum levels of PTH, calcium, phosphate, magnesium, creatinine, urea, hemoglobin, platelet count, age, and sex are shown in Table 4. Significant positive correlations were found between serum 25(OH)D levels and albumin, hemoglobin, and platelet count. There were significant negative correlations between the 25(OH)D concentration and INR, bilirubin, and ALP. There was no correlation between 25(OH)D and PTH, as well as calcium (corrected for albumin), phosphate, magnesium, parameters of renal function, age, or sex. Likewise, there was no correlation between serum PTH levels and any of the earlier-mentioned variables (not shown).

When multiple linear regression analysis was performed with 25(OH)D as the dependent variable and all other parameters with a *P* value of .15 or less as independent variables, the serum 25(OH)D level significantly and independently correlated only with INR (coefficient, −19.2; 95% CI, −35.1 to −3.4; $P = .018$) and albumin (coefficient, 1.06; 95% CI, 0.3–1.8; $P = .007$). This is not surprising because significant correlations existed between the indices of liver function. Pearson correlation coefficients were highly significant for albumin and bilirubin ($r = 0.524$; $P = .0001$), ALP ($r = 0.494$; $P = .001$), hemoglobin ($r = 0.700$; $P = .001$), and platelet count ($r = 0.461$; $P =$

Table 4. Results of Linear Regression Analysis in Patients With Noncholestatic CLD (n = 100) With Serum 25(OH)D as Dependent and Biochemical Markers of Liver and Renal Function, Serum PTH, Calcium, Hemoglobin, Platelet Count, Age, and Sex as Independent Variables

	Coefficient	95% CI	*P* value
Age	−0.3094	−0.733 to 0.115	.151
Sex	−0.6782	−11.340 to 9.984	.900
INR	−31.553	−44.120 to −18.987	<.001
Albumin level	1.520	0.971–2.069	<.001
ALT level	−0.003	−0.040 to 0.337	.862
AST level	−0.016	−0.193 to 0.160	.851
GGT	−0.017	−0.038 to 0.005	.128
Bilirubin level	−0.185	−0.277 to 0.092	<.001
PTH level	0.553	−1.462 to 2.569	.587
Calcium level	7.089	−42.481 to 56.659	.770
ALP level	−0.084	−0.134 to −0.034	<.001
Creatinine level	−0.208	−0.433 to 0.022	.076
Urea level	−0.339	−1.496 to 0.817	.562
Hemoglobin level	0.357	0.169–0.544	<.001
Platelet count	0.0826	0.003–0.126	.007

CI, confidence interval.

.001), as well as for INR and bilirubin ($r = 0.551$; $P = .0001$), ALP ($r = 0.334$; $P = .0007$), hemoglobin ($r = -0.563$; $P = .0001$), and platelet count ($r = -0.551$; $P = .0001$).

Table 5 summarizes the results of the logistic regression analysis regarding the degree of vitamin D deficiency (patients with serum 25[OH]D level >50 nmol/L used as a reference group). In noncholestatic CLD patients after adjustment for age and sex, moderate to severe vitamin D deficiency (serum 25[OH]D <25 nmol/L) predicts coagulopathy, hyperbilirubinemia, hypoalbuminemia, increased ALP concentration, anemia, and thrombocytopenia. Mild vitamin D deficiency (serum 25[OH]D 25-49 nmol/L) is predictive for the first 2 abnormalities whereas hypoalbuminemia and thrombocytopenia show borderline significance. In other words, there is a dose-response relationship between the serum 25(OH)D concentration and the presence of biochemical and hematologic abnormalities of CLD.

Taken together, these findings show that in CLD, serum 25(OH)D status is a significant predictor of liver injury, in particular for INR value, albumin level, bilirubin and hemoglobin concentrations, ALP activity, and platelet count.

Discussion

This study of 100 consecutive ambulatory noncholestatic CLD patients with a wide range of disease severity and diverse causes showed that the majority of these subjects (91%) had an inadequate vitamin D status. We showed that in CLD the prevalence and degree of vitamin D deficiency correlates with the severity and progression of liver disease, but secondary hyperparathyroidism is relatively uncommon and occurs in only 13% of those with vitamin D deficiency. Because the vitamin D endocrine system has important calciotropic and noncalciotropic functions, and vitamin D deficiency is easily preventable, heightened awareness is needed to ensure adequate vitamin D status in CLD patients.

Our data may indicate the significance of low vitamin D status both as a common complication of and a contributing factor to the pathogenesis of CLD. In our study, which describes a large diverse group of ambulatory noncholestatic CLD patients, the prevalence of vitamin D inadequacy (<80 nmol/L) was 100% in cirrhotic and 81.6% in noncirrhotic patients. The prevalence of vitamin D deficiency (<50 nmol/L) varied from 49% in noncirrhotic patients to 86.3% in cirrhotic patients. It was observed in all Child–Pugh class C patients, reflecting the severity of liver disease.

Low serum 25(OH)D concentrations have been reported in a variety of CLDs, especially in primary biliary cirrhosis[34,35] and before orthotopic liver transplantation,[15,23] but also in alcoholic and viral cirrhosis,[12,13,16,17,26,27,36] noncirrhotic CLD,[37,38] and hemochromatosis.[39] However, some researchers found no evidence of vitamin D insufficiency in cirrhosis,[20–22,28] noncirrhotic viral liver disease,[18,22] and hemochromatosis.[40] Furthermore, although in some studies serum 25(OH)D levels were reduced significantly in patients with decompensated cirrhosis,[17,25,27] in others no differences between Child–Pugh groups were observed.[24] Because of heterogeneity of diseases and patients, as well as variable methods and definitions used in the assessment of vitamin D status, direct comparison of our data and previous studies cannot be performed. However, our find-

Table 5. Predicting Liver Insufficiency and Hematologic Abnormalities in Patients With CLD From Degree of Vitamin D Deficiency: Logistical Regression Analysis, Adjusted for Age and Sex

	25(OH)D < 25 *nmol/L* (n = 28)			25(OH)D 25–49 *nmol/L* (n = 40)		
	OR	95% CI	*P* value	OR	95% CI	*P* value
INR > 1.1	20.1	4.9–81.7	.000	8.4	2.5–27.7	<.001
Bilirubin level ≥ 21 *μmol/L*	18.2	4.3–76.2	.000	3.7	1.0–12.8	.042
Albumin level ≤ 34 *g/L*	24.7	9.6–93.3	.000	4.0	0.79–16.7	.060
ALP level > 110 *U/L*	9.4	2.5–34.5	.001	2.1	0.7–6.4	.191
Hemoglobin level < 120 *g/L*	10.4	2.2–50.6	.004	3.4	0.8–14.0	.092
Platelet level < 150 $\times 10^9$/L	6.8	1.9–24.4	.003	3.2	0.9–11.0	.067
PTH level > 6.8 *pmol/L*	0.72	0.20–2.6	.017	0.44	0.13–1.5	.201

NOTE. Subjects with serum 25(OH)D level greater than 50 nmol/L constitute the reference group.
OR, odds ratio; CI, confidence interval.

ing of the high prevalence of vitamin D insufficiency/deficiency in noncholestatic CLD and its association with the severity of liver disease is in line with many previous studies.

Our study also showed that in noncholestatic CLD, vitamin D status correlates with and predicts in a dose-response manner liver function insufficiency such as increased prothrombin time, hypoalbuminemia, increased ALP activity, hyperbilirubinemia, and hematologic abnormalities (thrombocytopenia and anemia). This is an important A significant negative correlation between total bilirubin levels and bone mineral density in CLD was reported.[11,41]

The strong relationship between both the prevalence and degree of vitamin D insufficiency and the severity of CLD, especially Child–Pugh class, may indicate specific impairment of vitamin D metabolism in the liver. Indeed, impaired 25-hydroxylation of vitamin D related to the degree of hepatic dysfunction has been reported in patients with alcoholic cirrhosis.[12,34,42] In rats, bile duct ligation resulted in a 64% decrease in hepatic 25-hydroxylation of vitamin D.[43] In some studies, impairment of this enzymatic function was observed only in the advanced stages of CLD.[44] However, other studies claimed an adequate production of 25(OH)D even in advanced stages of CLD because after administration of oral or parental ergocalciferol or radiolabeled vitamin D to patients with cirrhosis and cholestasis, serum 25(OH)D concentrations become normal.[45–47] It appears that reduced vitamin D hydroxylation in the liver could not be considered as the only or universal mechanism of low serum 25(OH)D levels in CLD.

Although we found a strong association between serum 25(OH)D concentration and liver injury, this does not establish the relationship as causal. One would expect older patients to have lower 25(OH)D levels. However, there was no age difference in our series. Other possible factors contributing to vitamin D insufficiency in CLD may include the following: (1) reduced exposure to sunlight (patients with CLD and greater liver function abnormalities possibly spend less time outdoors), (2) dietary insufficiency (particularly in alcohol-related CLD), (3) malabsorption, (4) low levels of serum proteins that bind with vitamin D, (5) effects of medications (antiviral drugs, glucocorticoids, drugs affecting hepatic cytochrome P450 enzymes involved in vitamin D metabolism,[48] and (6) impaired cutaneous synthesis of vitamin D in jaundiced patients. One could speculate that in individual CLD patients, inadequacy in vitamin D status is determined by different pathogenic factors.

However, irrespective of factors involved in the development of vitamin D insufficiency, which is likely to be multifactorial in CLD, this abnormality should not be ignored. Vitamin D insufficiency is a well-recognized risk factor for osteoporosis in the general population[49,50] and increasingly is being recognized as a significant risk factor in a wide range of chronic inflammatory and autoimmune diseases (inflammatory bowel disease, rheumatoid arthritis, psoriasis, multiple sclerosis, diabetes mellitus), cancers (colon, prostate, breast), and metabolic disorders (metabolic syndrome, hypertension).[2,3,5] There are a number of ways in which vitamin D may influence hepatic injury, fibrosis, and tissue remodeling. Vitamin D and its derivates are potent regulators of cell proliferation, differentiation, and immunomodulation.[3] These effects include inhibition of certain matrix metalloproteinases (MMPs) and induction of their inhibitors,[30] suppression of proliferation of fibroblasts, and increased collagen production.[29] Vitamin D insufficiency is associated with increased circulating MMP-2 and -9, which is correctable by supplementation.[51] Hepatocytes produce the major MMPs and tissue inhibitors involved in liver extracellular matrix remodeling.[52] MMP-2 and -9 are of particular relevance to the liver because they are critically involved in the degradation of components of the basement membrane such as collagen IV and fibronectin, 2 main components of the space of Disse. Inhibition of MMPs protects from hepatic ischemic injury.[53,54] Therefore, low serum 25(OH)D may, at least in part, contribute to the progression of liver disturbance in CLD, and correction of vitamin D insufficiency may represent an important therapeutic target in anticirrhotic strategies for CLD. On practical ground, in CLD an evaluation of the 25(OH)D serum level is necessary and oral vitamin D should be administered to maintain a 25(OH)D level of 80 nmol/L or more. However, a large intervention study in CLD patients with inadequate vitamin D status is needed to find whether supplementation with vitamin D will reduce the decrease in liver function over time.

Our data that severity of CLD, especially progression of cirrhosis, parallels the reduction in serum levels of 25(OH)D but not PTH changes are similar to the observations of others.[15,23–25] No significant correlation was found between serum PTH and 25(OH)D levels in patients with end-stage liver disease.[55] Normal, low, or even undetectable levels of PTH were reported in primary biliary cirrhosis, other cirrhosis,[24] and before liver transplantation,[23] although some investigators found increased PTH levels in 3%[16]–42%[22] of cirrhotic and noncirrhotic patients. Interestingly, after liver transplantation an early transient increase in serum PTH levels without significant changes in serum 25(OH)D concentrations was reported.[23] Taken together, these observations do not support the view that reduced clearance capacity for PTH metabolites in the liver causes the PTH increase in advanced CLD.[22]

PTH secretion is controlled by vitamin D and calcium via the vitamin D receptor and calcium-sensing receptor, respectively. A negative relationship between serum 25(OH)D and serum PTH is a well-known physiologic phenomenon. The threshold of serum 25(OH)D when serum PTH starts to increase is about 75–80 nmol/L.[32,56,57] In our series 91 of 100 patients with CLD had 25(OH)D levels less than 80 nmol/L but only in 13 (14.3%) patients was the serum PTH concentration increased (>6.8 pmol/L). Moreover, of 68 subjects with vitamin D deficiency (25[OH]D, <50 nmol/L), secondary hyperparathyroidism was found in only 9 (13.2%) patients, although it was present in 3 of 9 patients with serum 25(OH)D levels higher than 80 nmol/L. The cause of normal to low PTH levels in the presence of vitamin D insufficiency and even severe deficiency observed in our study and other studies[23–25] is unclear.

In our study the absence of compensatory increases in PTH level cannot be explained either by the use of antiviral drugs, glucocorticoids, spironolactone, or by disturbances in calcium, phosphate, and magnesium levels. Possible explanations may include vitamin D–receptor gene polymorphism, which is associated with hypoparathyroidism in chronic renal failure,[58] and suppression of PTH secretion by L-amino acids that activate calcium-sensing receptor.[59] Pathophysiologic mechanisms contributing to the vitamin D–PTH paradox in CLD require further study.

The present study had some limitations because of its cross-sectional design. A prospective study with administration of vitamin D, correction of serum 25(OH)D levels, and reassess-

ment of liver function tests is highly desirable. Another limitation of this study was that vitamin D, calcium, and protein intake were not assessed and the results may be applicable only to white populations. Finally, none of our patients had overt steatorrhea, however, it should be noted that stool fat was not measured.

In conclusion, vitamin D inadequacy is very common in noncholestatic CLD patients and correlates with the severity of the disease. Therefore, we recommend that clinical guidelines for managing CLD should include the assessment of vitamin D status (by measuring serum 25[OH]D concentrations) in all patients and initiating vitamin D replacement when necessary.

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