Relationship of Serum Vitamin D with Liver Disease Severity and Bone Abnormalities in Cholestatic Children

Evangeline P. Capul, MD,[1] Germana Emerita V. Gregorio, MD, PhD[1] and Jarold P. Pauig, MD[2]

[1]Section of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, Philippine General Hospital, University of the Philippines Manila, Manila, Philippines
[2]Department of Radiology, Philippine General Hospital, University of the Philippines Manila, Manila, Philippines

ABSTRACT

Background. Vitamin D deficiency occurs in 10% to 36% of children with cholestasis. The relationship between serum vitamin D levels, severity of liver disease and bone abnormalities in children has not been extensively investigated.

Objective. To determine serum vitamin D levels and its association with liver disease severity and presence of radiographic rickets in children with cholestasis

Methods. Children aged 0–10 years with cholestasis underwent serum 25-hydroxyvitamin D levels (25-[OH]D) determination, radiographs of wrists and knees and liver function tests. Liver disease severity was evaluated using the Child-Pugh score. Radiographs were assessed using Thacher Rickets Severity Score. Data were analyzed using odds ratio and Spearman's correlation coefficient.

Results. We included 51 children [Mean (SD) age: 5 (6) months, 63% are males], mostly with biliary atresia (51%). Forty-seven (92%) had serum 25-(OH)D deficiency and four (8%) had insufficiency. Radiologic bone abnormalities were observed in 22 (43%) cases; specifically, rickets in 16 (31%). No association was observed with vitamin D levels and liver disease severity (OR 1.27, 95% CI 0.12–13.31) nor with rickets score (OR 0.07, 95% CI 0.004–1.37).

Conclusion. Majority of the children with cholestasis had vitamin D deficiency, with a third having radiographic findings of rickets. Serum vitamin D levels were not associated with liver disease severity or with rickets score.

Key Words: Vitamin D, rickets, cholestasis, bone disease, bone abnormalities

INTRODUCTION

Vitamin D deficiency occurs in patients with cholestasis, from 10% to 36% in children, to 92% in adults, regardless of the underlying cause of liver disease.[1-4] This may be secondary to dietary insufficiency, decreased availability of bile salts essential for the absorption of fat-soluble vitamins, defective hepatic conversion of vitamin D2 or D3 to the hydroxylated molecule or a reduced production of vitamin D-binding protein.[5]

Normally, exposure to sunlight is the main source of vitamin D, which is converted to 25-hydroxyvitamin D (25-[OH]D) in the liver, and then converted into the metabolically active form, 1,25-dihydroxyvitamin D (1,25-[OH]D) in the kidney.[1] The body stores of vitamin D are documented by the measurement of serum levels of 25-[OH]D,[6] which has a half-life of 2–3 weeks.[7] 1,25-dihydroxyvitamin D, the biologically active form, has a half-life of only 4–6 hours

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Corresponding author: Germana Emerita V. Gregorio, MD, PhD
Department of Pediatrics
Philippine General Hospital
University of the Philippines Manila
Taft Avenue, Manila 1000, Philippines
Email: gvgregorio@up.edu.ph

and therefore, the circulating levels are a thousand-fold less than 25-(OH)D.

There are limited reports on serum vitamin D levels in children with chronic liver disease. In a study of 48 infants aged less than 24 months with cholestatic liver disease in Iran, 22 (46%) had evidence of rickets on X-ray evaluation, 26 (54%) had vitamin D insufficiency (25-(OH)D 10-29 nM) and five (10%) had vitamin D deficiency (25-(OH)D <10 nM).[1] On the other hand, 24 children from Brazil with biliary atresia, with or without cholestasis, were noted to have low levels of vitamins A, D, and E as compared with healthy controls.[8] The results suggest that apart from malabsorption, the transport, metabolism and storage of fat soluble vitamins may also be affected in the presence of liver disease.

Overall, the presence of vitamin D deficiency, with disturbance of calcium and phosphate metabolism, predisposes patients with cholestastic liver disease to rickets, osteoporosis and fracture. In adults, all 32 patients with viral cirrhosis had low bone mineral density in the lumbar spine and femoral neck and 53% had evidence of osteoporosis.[9] Among 475 patients with primary biliary cirrhosis with varying severity of liver disease, 20% to 30% had osteoporosis and 6% to 14% had fractures.[10]

The Thacher Rickets Severity Scoring is an effective tool for assessing the presence and severity of rickets at diagnosis and on follow-up. The distal ulna can be utilized as the radiologic indicator for diagnosis and follow-up but the distal femur is a more reliable tool in cases with a severity score of 10 or those in which rickets is taking more than 3 months to heal.[11]

The relationship between serum vitamin D levels, severity of liver disease and presence of radiologic evidence of rickets in children has not been extensively investigated. This knowledge is important for timely institution of appropriate treatment including liver transplantation. Liver transplantation has been shown to improve bone disease in children.[1]

The objective of this study is to determine the serum vitamin D levels of children with cholestatic liver disease and correlate it with severity of liver disease and presence of radiographic bone abnormalities.

METHODS

Study Design and Setting

This was a cross-sectional study conducted from May 2016 to April 2017 at the Section of Pediatric Gastroenterology, Hepatology and Nutrition of the Philippine General Hospital (PGH), a government tertiary referral center in the country. This study was approved by the PGH Ethics Review Board.

Sample Size Computation

We estimated sample size based on assumptions of 10% vitamin D deficiency at 95% confidence level with the margin of error at 0.05. Assuming that 80 cases consult at the PGH for a period of 1 year and using the finite population computation, the sample size required is 51.[12]

Selection of Participants

We included patients aged 0 to 10 years old diagnosed with cholestatic liver disease in the study. Excluded were those with intake of vitamin D supplements and drugs that decrease vitamin D levels (anticonvulsants, steroids, rifampin, statins), presence of renal disease (proteinuria, hematuria, urinary tract infections), endocrine disorder (hypothyroid, hyperthyroidism, hypoparathyroidism, hyperparathyroidism), gastric or bowel resection, and vitamin D-deficient diets (ovo-vegetarians, vegans and those who have milk allergy and lactose intolerance).[13]

Study Procedure

The purpose and mechanics of the study were discussed with the parent or guardian and if possible, with the participant. We obtained informed consent from the parent or guardian and verbal assent from the participants older than 7 years. History-taking and physical examination were done and recorded by the principal investigator.

Study Parameters

Information on the presence or absence of jaundice, abdominal pain, abdominal distention, acholic stools and tea-colored urine was noted. Pertinent findings on physical examination were documented including the presence or absence of jaundice, ascites, hepatomegaly, splenomegaly, spider angioma and palmar erythema.

Biochemical findings. Laboratory tests were done as part of the initial assessment of the patient-serum levels of albumin, bilirubin (total, direct, indirect), transaminases and prothrombin time. All examinations were done at the UP-PGH Department of Laboratories using standard techniques (Fusion Vitros 5.1FS, New Jersey).

Serum 25-hydroxyvitamin D levels. During the blood extraction of the patient, an additional 5mL of blood was taken and serum was stored at -16º C freezer until recruitment of patients has been completed. All samples were processed at the PGH Radioisotope Laboratory for in vitro determination of 25-hydroxyvitamin D3 (25-OH-D3) and D2 (25-OH-D2) (Beckman Coulter Radioimmunoassay, California) by a research assistant who was blinded to the clinical information of the patient. Results were classified as vitamin D deficiency (<30 nmol/L or <12 ng/mL), vitamin D insufficiency (30-50 nmol/L or 12-20 ng/mL), vitamin D sufficiency (50-125 nmol/L or 20-50 ng/mL) and vitamin D toxicity (>125 nmol/L or >50ng/mL).[13]

Radiographic assessment. X-rays of both wrists and knees with anteroposterior and lateral views (Shimadzu Collimator Type R-20Jl Tokyo, Japan) were done at the PGH Department of Radiology. The plates were evaluated by a single-blinded radiologist using the Thacher Rickets

Severity Score, a 10-point scoring system for x-rays of bilateral wrists and knees formulated to assess the degree of cupping, metaphyseal fraying and the percentage of the affected growth plate. A score of more than 0 was considered abnormal.[14]

Disease severity. The Child-Pugh classification was utilized to evaluate the severity of liver disease.[6] Five variables, namely, the presence of ascites, degree of encephalopathy and the serum levels of bilirubin, prothrombin time and albumin were each scored from one to three and total score was used to assess the severity of illness. A score of 5–6 is classified as compensated liver disease (Class A); 7–9, moderate liver disease (Class B) and 10–15, severe or decompensated liver disease (Class C).[6]

Data Analysis

Statistical analysis was performed using Microsoft Excel. We computed means and standard deviations for continuous variables. The relationship of the serum vitamin D levels with the Thacher Rickets Severity Score and with severity of liver disease were assessed by using the odds ratio and Spearman Rho coefficient. A P value less than 0.05 was considered statistically significant.

RESULTS

Clinical and Biochemical Features

A total of 51 patients were included (Table 1). The mean age of patients was 5 months; with the youngest at 6 weeks old and the oldest at three years old. The majority (67%) were less than 6 months of age. Majority were males (63%). All 51 patients presented with jaundice after the first two weeks of life. The mean duration of jaundice before inclusion was 15 weeks, ranging from 1 week to 21 weeks. Majority of patients had biliary atresia (51%).

All patients had elevated bilirubin and alkaline phosphatase levels (Table 2). Aspartate and alanine transferase were elevated in 96% and 92% of patients, respectively. Twenty-one patients (41%) had prolonged prothrombin time (INR >1.2) and 19 (37%) had hypoalbuminemia (albumin <31 g/L).

On the basis of the clinical and biochemical findings, 36 (67%) patients were classified as Child-Pugh B (moderate liver disease) and 15 (23%) as Child-Pugh C (severe liver disease).

Table 1. Clinical features of patients with cholestatic liver disease (N=51)

Clinical feature	No. (%)
Mean (SD) age, months	5 (6.3)
Males	32 (63)
Mean (SD) duration of jaundice before diagnosis, weeks	15 (1)
Clinical features	
Acholic stools	40 (78)
Tea-colored urine	31 (61)
Abdominal distention	18 (35)
Ascites	18(35)
Hepatomegaly	47 (92)
Splenomegaly	29 (57)
Palmar erythema	7 (14)
Final diagnosis	
Biliary atresia	26 (51)
Neonatal hepatitis	22 (43)
Choledochal cyst	2 (4)
Neonatal sclerosing cholangitis	1 (2)

Serum 25-hydroxyvitamin D levels

Forty-seven (92%) patients had vitamin D deficiency (25-[OH]D <12 ng/mL) and four (8%) had vitamin D insufficiency (25-[OH]D=12–20 ng/mL) (Table 3). Among those with vitamin D deficiency, the duration of jaundice was less than two months in 18 of 21 (86%) cases and greater than two months in 29 of 30 (97%) patients (Fisher exact test P value = 0.29).

There is no association between the severity of liver disease and the serum vitamin D levels (OR 1.27, 95 % CI 0.12-13.32; P=0.84) (Table 4). With a Spearman rho (R) value of –0.18 and a two-tailed P-value of 0.19, there was no statistically significant correlation between the the serum vitamin D levels and Child-Pugh Score (Figure 1).

Radiographic assessment

Twenty-two patients had abnormal radiographic findings; sixteen patients had one bone involvement while six had two bones involved (Table 5). Of the 22 patients (43%) with radiographic abnormalities, the overall mean (SD) Thacher Rickets Score was 1.6 (0.9) (Figure 2A and

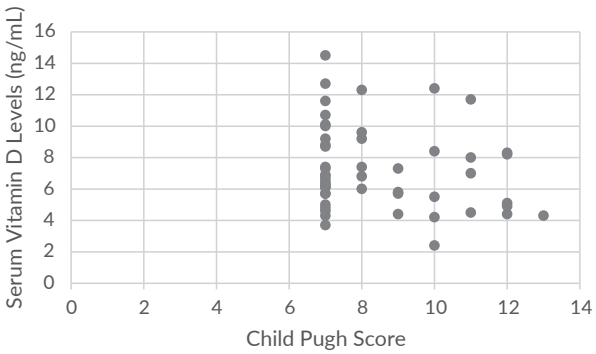

Figure 1. Association between serum vitamin D level and severity of liver disease.

Table 2. Biochemical features of patients with cholestatic liver disease (N=51)

Laboratory test	Mean (SD)	Normal Values
Total bilirubin	12.4 (5.2) mg/dL	0-2 mg/dL
Direct bilirubin	8.9 (3.7) mg/dL	0-0.9 mg/dL
Aspartate transferase	332.6 (266.7) IU/L	22-58 IU/L
Alanine transferase	187.2 (128.4) IU/L	11-39 IU/L
Alkaline phosphatase	664.9 (434.3) IU/L	32-91 IU/L
Albumin	34.0 (9.8) g/L	31-48 g/L
INR	1.3 (0.8)	0.8-1.2

INR, International normalized ratio

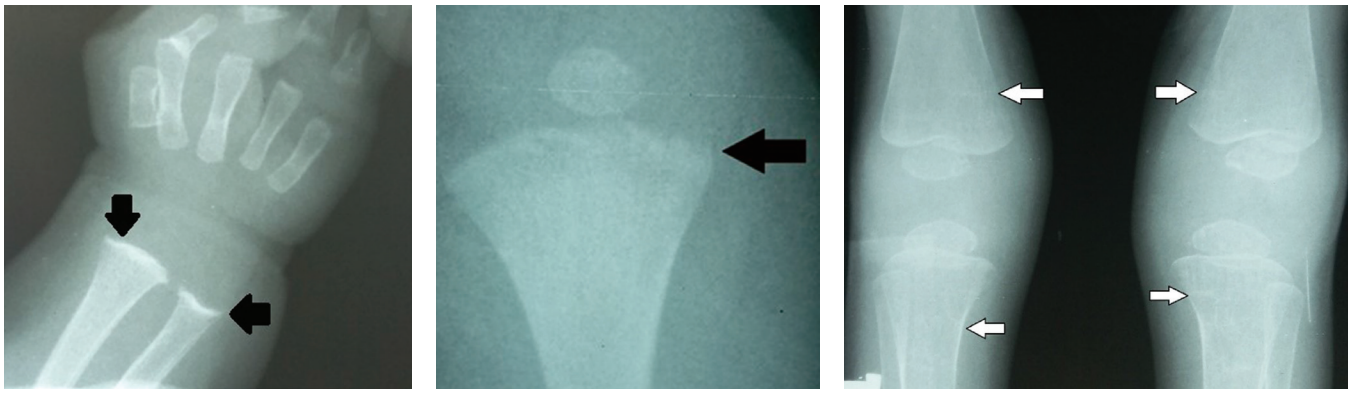

Figure 2. ***Radiographic findings.*** **(A)** 2-month-old male with concave and frayed metaphyseal margins of the radius and ulna *(black arrows)*. **(B)** Six-week-old female with partial lucency and disappearance of the smooth metaphyseal margins of the femur *(black arrow)*. **(C)** Incidental findings of bilateral stress fractures in the femur and tibia *(white arrows)* in a 1-year-old male.

2B). Three patients had fractures of the right radius, left proximal tibia, and bilateral femur and tibia (Figure 2C). No significant association was noted between the serum vitamin D levels and Thacher Rickets Score (OR 0.07, 95% CI 0.004-1.37; P=0.078; R=0.02, P=0.87] (Table 6) (Figure 3).

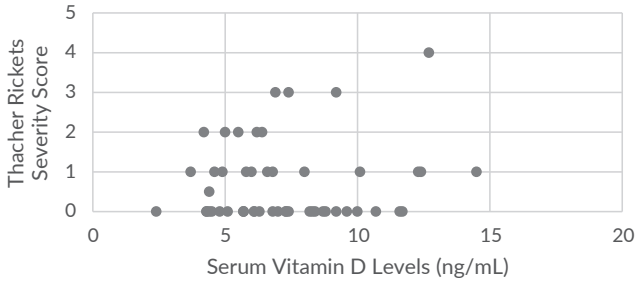

Figure 3. Association between serum vitamin D levels and Thacher Rickets Severity Score.

Table 3. Serum vitamin D levels of patients with cholestatic liver disease, based on duration of jaundice (N=51)

Duration of jaundice	Vitamin D levels	
	Deficiency (<12 ng/mL) n=47	Insufficiency (12–20 ng/mL) n=4
≥ 2 months	29	1
< 2 months	18	3

Fisher's exact test, P=0.29

Table 4. Serum vitamin D levels of patients with cholestatic liver disease, based on Child-Pugh classification (N=51)

Child-Pugh Classification	Vitamin D levels	
	Deficiency (<12 ng/mL) n=47	Insufficiency (12–20 ng/mL) n=4
Severe liver disease (C)	14	1
Moderate liver disease (B)	33	3

OR=1.27 [95% CI 0.12–13.31]; P=0.84

DISCUSSION

Cholestasis is defined as reduced flow of bile in the duodenum caused by the absence of intrahepatic bile ducts, an obstruction of the extrahepatic ducts or the failure of hepatocytes to secrete bile.[2] Metabolic bone disease that may occur in individuals with chronic liver disease is referred to as hepatic osteodystrophy.[10] Our study showed that vitamin D deficiency is present in majority of children with cholestatic liver disease. We also showed that vitamin D levels were not associated with the severity of liver disease, as measured by the Child-Pugh Classification, and the presence of rickets, as assessed by the Thacher Rickets Severity Score.

The high prevalence of vitamin D deficiency (92%) that we observed in the current study is considerably higher than that observed by Bastos from Brazil (36% or 5/22),[2] Argao

Table 5. Radiographic findings of patients with cholestatic liver disease (N=51)

Radiographic findings	No. (%)
Normal result	29 (57)
With one bone involved	16 (31)
Ulna	14
Radius	1
Femur	1
With two bones involved	6 (12)
Radius and ulna	5
Femur and ulna	1

Table 6. Serum vitamin D levels of patients with cholestatic liver disease, based on Thacher Rickets Severity Score (N=51)

Thacher Rickets Severity Score	Vitamin D levels	
	Deficiency (<12 ng/mL) n=47	Insufficiency (12–20 ng/mL) n=4
Abnormal (>0)	18	4
Normal (0)	29	0

OR=0.07 [95% CI 0.004-1.37]; P=0.078

from the United States (29% or 10/34)[15] and Mohammadi from Iran (10% or 5/48).[1] In the latter, however, 60% (29 of 48) also had vitamin D insufficiency.[1] It is unknown whether the disparity is due to the type of population investigated or the assay used. All studies were done in children with cholestasis, mostly with extrahepatic biliary atresia, but in different age groups; aged 2 months to 20 years (Brazil and American studies), aged 0 to 10 years (Iran study), and aged six weeks to three years (current study). We excluded patients who were on vitamin D supplements while the Brazil study included patients whether on oral vitamin D intake or not. Two studies used radioimmunoassay to measure 25-hydroxyvitamin D but the definition for the cut-off values for vitamin D deficiency was different: <9 ng/mL in Brazil study and <12 ng/mL in the current study. Both values were based on recommendations of the assay manufacturer. The U.S. study considered vitamin D deficiency as less than 15 ng/mL utilizing high performance liquid chromatography while Mohammadi used less than 10 ng/mL but it is unknown what assay was used and whether the patients investigated were on vitamin D supplements.[1,2,15]

Our study showed that vitamin D deficiency occurs even among patients whose duration of jaundice is less than two months and this predisposes to development of rickets, osteoporosis and fractures. It has been reported that rickets may be present as early as the first two months of life. It rapidly worsens with increasing age in children with chronic cholestatic liver disease as a result of impaired mineralization of growing bones.[15] To asses the bone abnormalities, we obtained radiographs of the wrists and knees and used the Thacher Rickets Severity Score, an objective and reproducible method to determine the presence of rickets. This system was previously used in the assessment and follow-up of patients with nutritional rickets. Our results revealed that of the 51 patients, 22 (43%) had radiographic bone abnormalities and 16 (31%) had evidence of rickets in at least one bone, mostly in the radius and ulna, which is consistent with the observation that small bones are usually the common site of rickets.[11] The prevalence of rickets that we observed is slightly lower than the 46% (22 of 48) reported by Mohammadi.[1] Of note is that we both found no association between the vitamin D levels and the presence of bone abnormalities. This supports the view that apart from the vitamin D deficiency, other factors such as impaired hepatic function resulting in protein matrix alteration, may contribute to hepatic osteodystrophy. Another factor is the low levels of osteocalcin, which have been documented in children with cholestasis. Osteocalcin is a hormone secreted by osteoblast and plays an active role in bone formation.[16] A reduction in hepatic production of insulin-like growth factor-I (IGF-I) has also been proposed to cause impaired development of bone mass even among pediatric patients with compensated liver disease.[17]

Our study also showed that there is no association between the vitamin D levels and the severity of liver disease. Patients with either moderate or severe liver disease, based on Child-Pugh classification, were noted to have vitamin D deficiency. Our results are similar to the investigation done in adults diagnosed with non-alcoholic steatohepatitis (NASH) in which plasma vitamin D levels were not associated with histological severity of the disease.[18] These patients, however, are not comparable with our study population as the mechanism of vitamin D deficiency is different. NASH leads to increased insulin resistance which increases adiposity. This, in turn, results in low vitamin D levels due to increased deposition in adipose tissue; vitamin D being lipid-soluble.[18] In contrast with the findings in our study, vitamin D levels inversely correlated with advanced liver damage among patients with primary biliary cirrhosis, a progressive cholestatic autoimmune disease. Vitamin D has been proposed to affect auto-antibody production and regulation of immune-modulating cells, including T-cells, cytokines and macrophages.

CONCLUSION

In conclusion, the present study showed that majority of children with cholestatic liver disease has vitamin D deficiency even among patients with recent onset of jaundice. Thus, vitamin D supplements should be given upon diagnosis. Bone abnormalities such as rickets are common and involves smaller bones. Serum vitamin D levels are not associated with severity of liver disease nor with the presence of bone abnormalities.

Recommendation

We recommend similar studies in other institutions with follow-up monitoring of vitamin D levels and radiographic imaging studies.

Statement of Authorship

All authors participated in data collection and analysis, and approved the final version submitted.

Author Disclosure

All authors declared no conflicts of interest.

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