Association between vitamin D level and patients with cholestasis

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ABSTRACT

Background: Estimate the prevalence of vitamin D deficiency among patients diagnosed with chronic cholestasis and investigates the association between their clinical manifestations and lab results of 25(OH) D levels.

Methods: A cross sectional study of 50 patients aged > 3 months up to 18 years, who had medical care in the Beni-Suef University hospital, Beni-Suef governorate, Egypt. All patients were fully investigated including routine labs in addition to 25- [OH] D levels and dual energy X-ray absorptiometry (DXA).

Results: Mean age was 6.5±4.5 years. Mean serum 25 (OH) D levels was 37.9±28.2; 30% of patients had 25 (OH) D <20 ng/ml and 26% had 25 (OH) D 20-<30 ng/ml. Low BMD was diagnosed in 73.1% of patients. The spine DXA BMD ranged between -5.4 and -1.4 (-2.9±0.9 Z-scores). A delayed milestone was reported in 32% of patients, and 56% had signs of rickets.

Conclusions: Deficiency of Vitamin D, signs of rickets and osteoporosis were evident in chronic cholestasis patients. No statistical significance was detected between 25- D [OH] levels and clinical, laboratory and radiological findings.

Keywords: Cholestasis, Vitamin D, Deficiency, Osteoporosis, BMD

INTRODUCTION

Cholestasis; a pathologic state of bile reduction or abnormal flow into bile ducts resulting in an elevated conjugated serum bilirubin and bile salts concentrations consequently mal-absorption of vitamin D takes place especially those unexposed to the sun due to their chronic illness. Biliary atresia (BA) is encountered in 35–41% of cases diagnosed with cholestasis; followed by idiopathic causes (13–30%), endocrinological and metabolic disorders (9–17%), while progressive familial intrahepatic cholestasis (PFIC) and preterm birth represent 10% for each. Other rare causes include: Alagille syndrome (AS) (2–6%), infectious diseases (1–8%), mitochondriopathy (2%), and biliary sludge (2%).

Fat-soluble vitamin D deficiency is associated with hypocalcemia, hypophosphatemia reflected clinically as rickets, osteomalacia and in severe forms tetany may take place. Chronic hepatic insult results in Osteo-dystrophy in 9-83% of cases secondary to Malabsorption of vitamin D; an essential component for bone metabolism which needs primarily conversion to 25[OH] D in liver and to its active form 1,25- dihydroxy vitamin D in kidney.

Vitamin D receptor (VDR) expressed in body cells, including hepatic cells, points involvement of vitamin D-mediated effects in other body systems not only the musculoskeletal; resulting in regulation of calcium level in blood, cellular differentiation and xenobiotics detoxification in addition to innate-adaptive immunity modulation. Vitamin D deficiency secondary to chronic cholestasis is not the principal cause of osteopenia.
Alterations to bone mineral and vitamin D absorption; results in abnormal protein matrix leading to a metabolic bone disease. Patients with chronic cholestasis with corrected vitamin D levels continue to have metabolic bone diseases reinforcing the idea of other factors contributing to osteopenia. Scarcely literature is available for vitamin D levels in children and adolescent age groups diagnosed to have chronic cholestasis in Egypt.

The aim of this study is to assess vitamin D deficiency in children with cholestasis in Egypt and to investigate its association with other developmental milestones.

METHODS

Study design and setting

A cross-sectional study was conducted from June to October 2016 for 50 patients with cholestasis; age ranged from 1.1 to 17 years who had medical care in the Beni-Suef University hospital, Beni-Suef governorate, Egypt

Exclusion criteria

Patients with non-cholestatic chronic liver diseases; patients with concomitant HCV, HBV, HIV, hepatic or extra hepatic malignancies, inflammatory bowel disorders, celiac disease, and history of total parenteral nutrition during the previous 3 months.

Study patients were subjected to:

1- Full history taking: date of diagnosis, consanguinity, history of developmental delay and/or bone fractures.

2- Complete physical examination for: teeth development, bone deformities, scratch marks and clubbing in addition to abdominal examination to assess liver, spleen and presence or absence of ascites. For children < 2 years of age; height was measured by horizontal anthropometry and weight was assessed by a digital weigh scale.

3- Laboratory investigations:

   a. Liver function tests: Total and direct bilirubin, ALT, AST, GGT, albumin, alkaline phosphatase (ALP), PT, PTT and INR.

   b. Electrolytes: calcium and phosphate. Serum calcium levels were corrected for albumin concentration.

Vitamin D; 25(OH) level was tested using batched electrochemiluminescence immunoassay (Elecys 25(OH)D total assay, Roche diagnosis, Basel, Schweiz) and readings were categorized as:

- Normal (>30 ng/ml).
- Insufficient (20-30 ng/ml).
- Deficient (<20 ng/ml).

Later, 26 patients had total body and spine dual energy X-ray absorptiometry (DXA).

Patients with BMD- Z scores <2 and history of bone fractures or fissures were considered having osteoporosis.

Ethical considerations

Study protocol approval prior to implementation of the research was obtained from Faculty of Medicine, Beni-Suef University Research Ethics Committee. Parents were informed about the study purpose and its consequences, confirming confidentiality of data. A signed informed consent was a pre-requisite for the study.

Statistical analysis

Data collected were coded, analyzed by statistical package for social science, (SPSS) version 20; processed and tabulated. Frequency distribution, percentage and descriptive statistics including mean±SD were calculated. Chi-square test, t-test and correlations were performed when indicated. Spearman Rho correlation was used. P values of less than 0.05 were considered significant.

RESULTS

This study included 50 patients diagnosed with cholestasis who had medical care in the Beni-Suef University hospital, Beni-Suef governorate, Egypt with a mean age of 6.5±4.5 years (range: 1.1-17) and mean duration of cholestasis of 3.6±3.2 years (range: 0.3-12). History of consanguinity was reported in 70% of cases (Table 1). Clinical manifestations and their association with 25(OH) D levels are shown in (Table 2) Delayed musculoskeletal milestones was reported by 16 (32%) patients.

Teeth caries was diagnosed in 50% of the studied group, 6 (12%) had a history of bone fissures and 6 (12%) had history of bone fractures. Rickets was clinically diagnosed in 28 (56%) patients; 53% had inappropriate 25 (OH) D levels. Only one child with deficient 25 (OH) D levels was diagnosed with bone deformity.

Bleeding tendency was the most recorded sign; 26 (52%) of patients had bleeding gums or epistaxis or bleeding per rectum and/or hematemesis. Being easily bruised was also reported in 14 (28%) patients. Besides, palmer erythema was detected in 42 (84%) patients and clubbing in 30 (60%) patients. Hepatosplenomegaly, ascites and lower limb edema were also common signs in our patients.

Mean total and direct bilirubin levels in patients were much higher than normal ranges with 37(74%) of them having hyperbilirubinemia with elevated liver enzymes. Hypoalbuminemia was detected in 48% of patients. Positive correlation was only found between serum
calcium levels and 25(OH) D levels (Table 3). The mean total body DXA BMD ranged between -5.5 and -1.1 (-2.6±1.1 Z-scores) with 73.1% of examined patients showing low BMD. Spine DXA BMD ranged between -5.4 and -1.4 (-2.9±0.9 Z-scores) and 84.6% of them had below normal BMD. Only 5 (19.2%) children had both below average Z-scores and positive history of fissures or fractures and were diagnosed with osteoporosis (Table 4). Vitamin D supplementation did not improve BMD (p>0.05).

### Table 1: Patients characteristics and their association with 25(OH) D levels.

| Patients characteristics | Deficient n=15 (%) | Insufficient n=13 (%) | Normal n=22 (%) | Overall n=50 (%) | P value |
|--------------------------|--------------------|-----------------------|-----------------|------------------|---------|
| Age (mean±SD) years      | 7.1±5.0            | 6.7±4.9               | 6.0±4.1         | 6.5±4.5          | 0.723   |
| Duration (mean±SD) years | 4.4±3.6            | 2.7±2.4               | 3.6±3.3         | 3.6±3.2          | 0.413   |
| Weight <3rd percentile   | 5 (29.5)           | 3 (17.6)              | 9 (52.9)        | 17 (34.0)        | 0.554   |
| Height <3rd percentile    | 7 (43.8)           | 4 (25.0)              | 5 (31.2)        | 16 (32.0)        | 0.421   |
| Consanguinity             | 13 (37.1)          | 8 (22.9)              | 14 (40.0)       | 35 (70.0)        | 0.240   |

### Table 2: Clinical manifestations and their association with 25(OH) D levels.

| Clinical assessment              | Deficient n=15 (%) | Insufficient n=13 (%) | Normal n=22 (%) | Overall n=50 (%) | P value |
|---------------------------------|--------------------|-----------------------|-----------------|------------------|---------|
| Developmental delay*            | 3 (18.8)           | 3 (18.8)              | 10 (62.4)       | 16 (32.0)        | 0.192   |
| Teeth hyperpigmentation         | 2 (100.0)          | 0 (0.0)               | 0 (0.0)         | 2 (4.0)          | 0.088   |
| Teeth caries                    | 7 (28.0)           | 6 (24.0)              | 12 (48.0)       | 25 (50.0)        | 0.850   |
| Bone fissure                    | 1 (16.7)           | 0 (0.0)               | 5 (83.3)        | 6 (12.0)         | 0.102   |
| Bone fracture                   | 3 (50.0)           | 1 (16.7)              | 2 (33.3)        | 6 (12.0)         | 0.518   |
| Bone deformity "foot"           | 1 (100.0)          | 0 (0.0)               | 0 (0.0)         | 1 (2.0)          | 0.304   |
| Signs of rickets                | 7 (25.0)           | 8 (28.6)              | 13 (46.4)       | 28 (56.0)        | 0.678   |
| Night blindness/photophobia     | 1 (50.0)           | 1 (50.0)              | 0 (0.0)         | 2 (4.0)          | 0.437   |
| Bleeding tendency**             | 7 (26.9)           | 6 (23.1)              | 13 (50.0)       | 26 (52.0)        | 0.673   |
| Easily bruising                 | 5 (35.7)           | 3 (21.4)              | 6 (42.9)        | 14 (28.0)        | 0.830   |
| Jaundice                        | 15 (34.1)          | 10 (22.7)             | 19 (43.2)       | 44 (88.0)        | 0.164   |
| Pale stool                      | 0 (0.0)            | 1 (33.3)              | 2 (66.7)        | 3 (6.0)          | 0.498   |
| Dark urine                      | 14 (93.3)          | 7 (53.8)              | 15 (68.2)       | 36 (72.0)        | 0.059   |
| Pruritus                        | 8 (53.3)           | 6 (46.2)              | 15 (68.2)       | 29 (58.0)        | 0.872   |
| Scratch marks                   | 5 (33.3)           | 4 (30.9)              | 8 (36.4)        | 17 (34.0)        | 0.943   |
| Palmer erythema                 | 14 (38.9)          | 9 (21.4)              | 19 (45.2)       | 42 (84.0)        | 0.205   |
| Eczymosis                       | 1 (25.0)           | 1 (25.0)              | 2 (50.0)        | 4 (8.0)          | 0.964   |
| Hematoma                        | 0 (0.0)            | 0 (0.0)               | 0 (0.0)         | 0 (0.0)          | ...     |
| Petechiae                       | 0 (0.0)            | 1 (100.0)             | 0 (0.0)         | 1 (2.0)          | 0.234   |
| Loss of subcutaneous fat        | 0 (0.0)            | 0 (0.0)               | 2 (100.0)       | 2 (4.0)          | 0.266   |
| Clubbing                        | 12 (40.0)          | 5 (16.7)              | 13 (43.5)       | 30 (60.0)        | 0.081   |
| Palpable liver                  | 12 (29.3)          | 11 (26.8)             | 18 (43.9)       | 41 (82.0)        | 0.951   |
| Palpable spleen                 | 11 (30.6)          | 8 (22.2)              | 17 (47.2)       | 36 (72.0)        | 0.600   |
| Ascites                         | 1 (14.2)           | 3 (42.9)              | 3 (42.9)        | 7 (14.0)         | 0.458   |
| Lower limb edema                | 1 (33.3)           | 1 (33.3)              | 1 (33.3)        | 3 (6.0)          | 0.626   |

### Table 3: Different labs and their association with 25(OH)D levels.

| Labs                        | Abnormal levels (n=50,%), Range, Mean ±SD | Normal Ranges | Correlation with 25(OH)D | P value |
|-----------------------------|--------------------------------------------|---------------|--------------------------|---------|
| Total bilirubin (mg/DL)     | ‡37 (74.0), 0.2 - 31, 6.2±6.9              | 0.6-1.4       | -0.213                   | 0.137   |
| Direct bilirubin (mg/DL)    | ‡42 (84.0), 0.1 - 13, 3.2±3.4              | 0.2-0.8       | -0.265                   | 0.063   |
| ALT (IU/L)                  | ‡40 (80.0), 17.7 - 477.7, 111.7±94.1       | 7-56          | -0.178                   | 0.215   |
| AST (IU/L)                  | ‡44 (88.0), 24.6 - 823.5, 166.0±161.7      | 10-40         | -0.087                   | 0.547   |
| GGT (IU/L)                  | ‡40 (80.0), 4 - 664, 132.4±150.2           | 0-45          | 0.170                    | 0.238   |
| ALP (IU/L)                  | ‡24 (48.0), 237 - 7468, 1365.3±1556.1      | 44-147        | -0.113                   | 0.435   |

Continued.
One way ANOVA revealed that patients categorized as deficient 25(OH)D had significantly lower total body BMD than both insufficient and normal groups (p=0.009).

Causes of cholestasis were distributed as follows: familial progressive intrahepatic cholestasis 22/50 (44%), Wilson disease, autoimmune hepatitis, and glycogen storage disease 6/50 (12%) for each, failed KASAI operation 5/50 (10%), alagille syndrome 3/50 (6%), and cryptogenic cirrhosis 2/50 (4%). Laboratory vitamin D level estimation among the participants revealed that 30% had Vitamin D deficiency (<20 ng/ml); 26% had insufficient level (20-30 ng/ml), and 44% had normal vitamin D level.

| Labs                        | Abnormal levels (n=50, %) | Range   | Mean ±SD | Normal Ranges | Correlation with 25(OH)D | P value |
|-----------------------------|---------------------------|---------|----------|---------------|--------------------------|---------|
| Albumin (g/dL)              | ↑24 (48.0)                | 1.9 - 4.9 | 3.5±0.9 | 3.8-5.1       | 0.139                    | 0.336   |
| PT (s)                      | ↑41 (82.0)                | 11 - 32  | 15.8±4.4 | 11-13.5       | -0.133                   | 0.356   |
| INR (s)                     | ↑30 (60.0)                | 1 - 3.5  | 1.3±0.5  | 0.8-1.1       | -0.078                   | 0.589   |
| Calcium (mg/dL)             | ↑19 (38.0)                | 6.8 - 12.3 | 9.1±1.0 | 8.5-10.5      | 0.401                    | 0.006*  |
| Phosphate (mg/dL)           | ↑16 (32.0)                | 1.5 - 9.6 | 4.9±1.8  | **           | 0.145                    | 0.348   |
| Corrected calcium (mmol/L)  | ↑5 (10.0)                 | 7.9 - 10.7 | 9.5±0.7 | 2.1-2.6       | -0.378                   | 0.149   |
| 25 (OH)D (ng/ml)            | ↓13 (26.0)                | 4.5 - 106 | 37.9±28.2 | ***          | ----                     | ----    |

Table 4: Total body and spine DXA and their correlation with 25(OH)D levels.

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**DISCUSSION**

Cholestasis in addition to other factors predisposing to vitamin D deficiency and bone disease are well recognized complications of “cholestatic” liver disease.15

This cross sectional study conducted on 50 children revealed that the most common cause of cholestasis was progressive familial intrahepatic cholestasis (PFIC) reported among 22 patients (44%), higher than the reported 12%-27% results of others.16,17 This might be due to population and study tools difference. Other causes reported were Wilson disease, autoimmune hepatitis and glycogen storage disease (12% each). Contrary to others who reported that neonatal hepatitis is the commonest intrahepatic causes of cholestasis.17,18

In this study, 56% of patients were found to have hypovitaminosis D a finding that is higher than similar studies with reported prevalence of 25%-36% in patients with chronic hepatopathy and cholestasis.16,19 Vitamin D deficiency was reported in 30% < 20ng/ml) and 26% had insufficient levels (20-30 ng/ml). Findings are less than the reported 92.4% among 109/118 patients with vitamin D deficiency secondary to liver diseases not only cholestasis which disrupt activation of vitamin D in liver.20,21

Vitamin D deficiency leads to progressive liver diseases due to down-regulation of bile acid transport and metabolism, in addition to the loss of anti-inflammatory and immuno-modulatory effect, enhancing the damaging effect of cholestasis with bile ducts ruptures.22,23

In the current study vitamin D levels were not significantly different in patients with signs of rickets (p=0.678), a finding similar to a study conducted among 48 children with liver cholestasis from Tehran medical center, reflecting the role of other factors in bone health in children with cholestasis.17 Mean level of calcium reported was 9.1±1.0 with a statistical significance difference between calcium and 25 (OH)D levels, which is in agreement with results from the same center (r=0.5, p=0.001) highlighting the role of vitamin D deficiency in bone diseases.17,20

Bone densitometry was performed for 26 patients; low total BMD was reported in 73.1% and 84.6% had low spine BMD with no statistically significant differences when correlated with 25 OH vitamin D differences (p=0.088 and 0.52 respectively) findings which are in accordance with a similar study for 20 patients 3–18 years of age with chronic liver cholestasis who had decreased total bone mass and contradicts earlier reports showing that children with cholestasis had low BMD and osteoporosis with vit.25(OH)D deficiency (<20 ng/ml).24,25,32 From the present study it’s concluded that children suffering from chronic cholestasis with deficient 25(OH)D had significantly lower total body BMD than both insufficient and normal groups. Vitamin D
screening, monitoring and bone health scanning should be encouraged for this group of patients. Proper vitamin D dose intake and adequate compliance is mandatory to minimize risks of its deficiency.

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the Institutional Ethics Committee

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