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Bone Mineral Status in Children With Cholestatic Liver Diseases

[1]Ahmed A. Talaat, [2]M.S. El Raziky, [2]M.S. Haroun, [1]Hassan M. Salama, [3]S. El Tookhy and [1]Hasanein M. Hasanein

[1]Pediatric Department, Medical Division, National Research Centre (NRC), Cairo, Egypt.
[2]Pediatric Department, Hepatology Clinic, Cairo, University, Egypt.
[3]Clinical Pathology Department, Medical Division, NRC, Cairo, Egypt.

Abstract: Cholestasis can be defined as the accumulation of substances normally excreted in bile (e.g. bilirubin, bile acids, and cholesterol). The process occurs as a result of impaired bile formation by the hepatocytes or from obstruction to the flow of bile through the intra hepatic and extra hepatic biliary tree. The aim of this study was to make laboratory assessment of bone mineral status and detection of the impact of different biliary diseases, its severity, duration and applied management on these values. The present study included forty patients, twenty four males (60%) and sixteen females (40%) who had cholestatic liver diseases for more than 3 months and followed up in the hepatology clinic, Cairo University and twenty healthy age and sex matched controls. The mean for age was 2.1 years ± 1.22 among cholestatic patients and the duration of illness was 1.94 ± 0.97 years at diagnosis. The most common cause was neonatal hepatitis (n = 10, 25%). The most common clinical finding was hepatomegally (90%). Cases had a significantly lower level of vitamin D and calcitonin compared to controls, P = (0.005, 0.006) respectively. Vitamin D deficiency was more in fourteen patients who were not compliant to treatment. There was no significant difference between cases and controls regarding PTH level. Also positive correlation was present between serum calcium level and 25 (OH) vitamin D (r = 0.5, P = 0.001) and a negative one between parathyroid hormone and serum calcium level (r = 0.4, P = 0.01). We conclude that children with cholestatic liver diseases are liable to develop vitamin D and calcium deficiency which can lead to bone complications. Incompliance to treatment especially vitamin D doses was common in 40% of patients which increases the incidence of these complications.

Key words: Cholestatic liver disease- osteoporosis- 25(OH) vitamin D- serum calcium level.

INTRODUCTION

Cholestasis can be defined as a pathologic state of reduced bile formation or flow, in which substances normally excreted into bile are retained where the serum concentrations of conjugated bilirubin and bile salts are the most commonly measured (Strople *et al*., 2009). The overall incidence of neonatal liver disease manifesting clinical or biochemical evidence of cholestasis may be as high as 1 in 2500 live births (Balistreri, 2002).

The most common cholestatic liver disease in infancy is biliary atresia, which has an incidence of 1 in 10,000 to 20,000. Other common diseases of infancy include neonatal hepatitis, Alagille syndrome and progressive familial intra-hepatic cholestasis (PFIC) syndromes, which initially may compromise nutrition because of cholestasis with an attendant decrease in intraluminal bile acid content, resulting in fat and fat soluble vitamins malabsorption (Heubi *et al.*, 2008).

Neonatal jaundice is considered to be a frequent and early presenting feature of liver disease during early life rather than a late manifestation of advanced disease. Other clinical manifestations such as hepatosplenomgally, abdominal distension, failure to thrive, pruritis, steatorrhea, metabolic bone disease (osteopenia-osteoporosis) and bleeding per orifices, all are considered to be classic symptoms and signs that can be seen in cholestatic liver patients (Andrew *et al*., 2007).

Osteopenia is a recognized complication of cholestatic liver diseases, usually ascribed to metabolic bone diseases such as osteomalacia and osteoporosis, with prevalence from 10 to 56%, depending on the nature of liver disease. The pathogenesis of bone disease in children with cholestasis is not completely understood. There has been disagreement regarding the relative importance of osteomalacia versus osteoporosis as the factors leading to osteopenia of liver disease. Many studies are done to determine the exact mechanism of osteopenia and its pathogenesis, which proved to be multifactorial in origin (Klein *et al*., 2002).

Over the last two decades, better histomorphometric techniques have made it clear that the main bone abnormality in chronic liver disease, cholestatic or hepatocellular, is osteoporosis and that osteomalacia is very rare. Most histomorphometric studies have found osteoporosis in CLD to be of a low bone turnover type with reduced osteoblast function, and measurements of biochemical markers of bone metabolism, such as osteocalcin, parathyroid hormone have confirmed these findings (Diamond *et al.,* 2009). The rational for evaluation and

Corresponding Author: Ahmed A. Talaat, Pediatric Department, Medical Division, National Research Centre (NRC), Cairo, Egypt.

management of osteoporosis is prevention of the clinical morbidity of pain and immobility caused by fracturing. Most fracturing in liver patients occurs after liver transplantation, but pretransplant osteoporosis is the main risk factor for post transplant fracturing; therefore its understanding and subsequent management are important (Compston and Thompson, 2007).

Many different etiologic factors contribute to the occurrence of osteoporosis in liver disease, and these vary according to the type, severity and progression of liver disease (Hay, 2003). Also, cholestatic liver disease in children negatively affects nutritional status, growth, and development which all lead to increased morbidity and mortality (Cooke *et al*., 2008).

Aim of the Work:

To assess the bone mineral status in children with cholestatic liver diseases and to detect the impact of different biliary diseases, its severity, duration and applied management on these bone mineral laboratory values.

SUBJECT AND METHODS

Forty patients were included in the study; twenty four males and sixteen females, their age ranging from three months to ten years attending regular follow up at the Hepatology clinic, Cairo University, Pediatric hospital. Twenty healthy age and sex matched children served as controls.

1- Inclusion Criteria:

All cholestatic patients with age ranging from 3 months to 10 years were included in the study. No specific consideration as regard sex.

2- Exclusion Criteria:

Patients with chronic liver diseases due to other causes rather than cholestasis were excluded from the study

All patients in the study were subjected to the following:

1. full history taking with special emphasis on vitamin D replacement and the dose taken, manifestations of vitamin D deficiency such as broadening, delayed walking and fractures.
2. Complete physical examination as regard manifestations of cholestasis.
3. Laboratory investigations which included:
   a. serum calcium, phosphorus, magnesium, alkaline phosphatase, alanine aminotransferase (ALT), aspartate trasaminase (AST), glutamyle transpeptidase, bilirubin, serum albumin, prothrombin time, partial thromboplastin time and international normalized ratio, were taken from patients files.
   b. Human intact 25(OH) vitamin D was determined quantitatively in serum using immunoenzymetric assay- 25(OH) vitamin D direct ELIZA kit of immuno-diagnostic (Australia).
   c. Human intact parathyroid hormone was determined quantitatively in serum using immunoenzymetric assay of hPTH-EASIA of DIA.
   d. Human intact calcitonin hormone was determined quantitatively in serum using immunoenzymetric assay. CT-U.S- EASIA of DIA source (Belgium).

Data Analysis:

All data were statistically described in terms of range, mean, standard deviation (SD)-frequencies (number of cases) and relative frequencies (percentage) when appropriate. Comparison of quantitative variables was done using student t-test for independent samples when normally distributed and Mann Whitney u test for independent samples when not normally distributed. For comparing categorical data, chi square test (x) was performed. Exact test was used when the expected frequency was less than 5. A probability value (P value) <0.05 was considered to be statistically significant. All statistical calculations was done using computer programs-micro soft excel and SPSS (statistical package for the social science-version 2010).

Results:

This study included 40 cholestatic patients who were on regular follow up at Hepatology clinic; their age ranging from 3 months to 10 years, with a mean of 2.13 years ± 1.22 SD and a duration of illness 3 months to 5.5 years with a mean of 1.94 years ± 0.97 SD. Patients were 24 males (60%) and 16 females (40%) with 20 healthy, age and sex matched controls. The clinical data of the patients are shown in table 1.

The most common causes were neonatal hepatitis (n = 10, 25%), extrahepatic biliary atresia (n = 9, 22.5%), cholestasis with normal γ-GT (n = 9, 22.5%), progressive familial intrahepatic cholestasis (PFIC) (n = 5, 12.5%), Alagille syndrome (n = 4, 10%), ARCI syndrome (n = 2, 5%) (angiodysplasia, renal dystrophy, cholestasis and icthiosis) and benign recurrent intrahepatic cholestasis (BRIC) (n = 1, 2.5%). Figure 1.

Table 1: Descriptive statistics of clinical data of studied patients.

Variable	Number	Percentage (%)
Dark colored urine	17	42.5
Clay colored stool	10	25
Delayed walking	4	10
Delayed dentition	0	0
Signs of vit D deficiency(rickets)	0	0
Pruritis	14	35
Hepatomegally	36	90
Splenomegally	26	65
Hepatosplenomegally	26	65
Ascitis	5	12.5
Lower limb edema	0	0
Compliance of the treatment	26	65
Fractures	0	0
Bleeding tendency	0	0

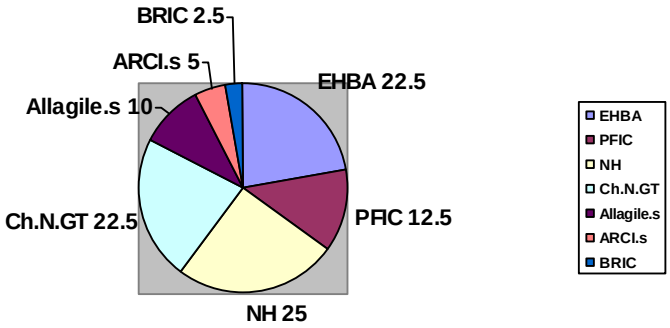

Fig. 1: Percentage of different disease etiology among the studied patients.

The laboratory data of the patients involved in the study are included in table 2. A comparison was done between cases and controls as regards serum level of 25 (OH) vitamin D, calcitonin and parathyroid hormone (PTH) as shown in table 3. There was a significant decrease in the serum level of vitamin D in patients (with a mean of 44.7nmol/l) compared to controls (with a mean of 136.9nmol/l), P value was significant P = 0.005. Also there was a significant decrease in the serum level of calcitonin in cases (with a mean of 7.4 pg/ml) compared to controls (with a mean of 25.1pg/ml), P value was significant P = 0.006. There was no significant difference between cases and controls regarding the serum level of parathyroid hormone.

Table 2: Descriptive statistics of the laboratory data of studied patients.

Variable	Min.	Max.	Mean ± SD
Vit D (nmol/l)	22.60	101.00	46.65±19.89
Calcitonin (pg/ml)	5.30	24.80	11.21±4.95
PTH (pg/ml)	10.50	91.10	29.28±19.13
Calcium (mg/dl)	6.80	10.20	8.26±0.79
Phosphorus (mg/dl)	1.80	5.60	3.46±0.71
Alkaline phosphatase (IU/l)	247	1675	559.5±273
Magnesium (meq/l)	1.4	3	2.14±0.40
Albumin (g/dl)	2.2	4.60	3.53±0.57
T bilirubin (mg/dl)	2.80	24.9	10.37±4.31
D bilirubin (mg/dl)	1.5	11.6	5.76±2.44
PT (seconds)	12	49.20	15.59±5.75
PC(percentage)	85	100	96.47±3.21
PTT(seconds)	28.50	71.4	42.37±7.01
INR	1	1.5	1.04±0.08
ALT (IU/ml)	40	546	125.6±106.3
AST (IU/ml)	21	640	101.5±100.1

Table 3: Comparison between cases and controls regarding the biochemical data.

Variable	Cases Mean ± SD N = 40	Control Mean ± SD N = 20	P -value
Vit D (nmol/l)	44.7±18.4	136.9±34.8	0.005*
Calcitonin (pg/ml)	7.4±1.5	25.2±7.6	0.006*
PTH (pg/ml)	25.8±13.48	25.57±13.37	0.957

P -Value is significant if P≤ 0.05*

The mean level of different laboratory values was determined for different disease etiology. We found that the mean serum calcium level was reduced in both biliary atresia and PFIC patients (P = 0.04) and the mean level of vitamin D was reduced in biliary atresia, Alagille syndrome and benign recurrent intrahepatic cholestasis patients (P = 0.02).

In this study, patients were classified into 2 groups:

1. Group (1) included 26 patients who were on regular vitamin D supplementation representing 60% of all cases.
2. Group (2) included 14 patients who were not on regular vitamin D supplementation representing 40% of cases.

Table 4: Biochemical data of studied cases in relation to dose of Vitamin D.

Variable	Group 1 Mean ± SD N = 26	Group 2 Mean ± SD N = 14	P -value
Mean of Vitamin D (nmol/l)	51.2	37.1	0.03*
Calcitonin (pg/ml)	11.52±5.11	10.37±4.99	1.33
PTH (pg/ml)	31.81±36.25	26.06±24.22	6.47
Mean of Calcium (mg/dl)	8.5	7.9	0.03*
Phosphorus (mg/dl)	3.28±0.48	3.79±0.933	0.25
Alkaline phosphatase (Iu/l)	543.2±260.1	593.2±303.5	81.11
Magnesium (meq/l)	2.11±0.465	2.22±0.294	0.07
Albumin (g/dl)	3.43±0.55	3.68±0.53	0.141
T bilirubin (mg/dl)	10.41±4.75	9.92±3.88	1.04
D bilirubin (mg/dl)	5.54±2.47	6.04±2.53	0.676
PT (seconds)	16.26±7.01	14.29±1.66	0.446
PC (percentage)	96.42±3.38	96.21±2.86	0.764
PTT(seconds)	43.83±7.18	40.34±5.22	1.39
INR	1.04±0.11	1.05±0.045	0.133
ALT (Iu/ml)	101.01±47.5	165.35±165.1	44.13
AST (Iu/ml)	79.28±38.03	138.42±155.3	41.50

In table 4, group (1) cases had a significantly higher level of serum vitamin D and calcium compared to group (2) cases (P = 0.03) for both. Figure 2, 3 respectively.

All the other laboratory findings did not have any significant difference between the two groups.

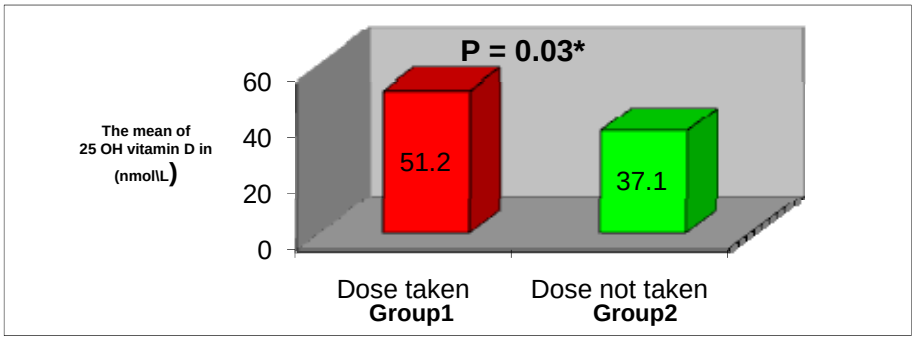

Fig. 2: The mean of serum 25 (OH) vitamin D level in the two groups of patients involved in the study.

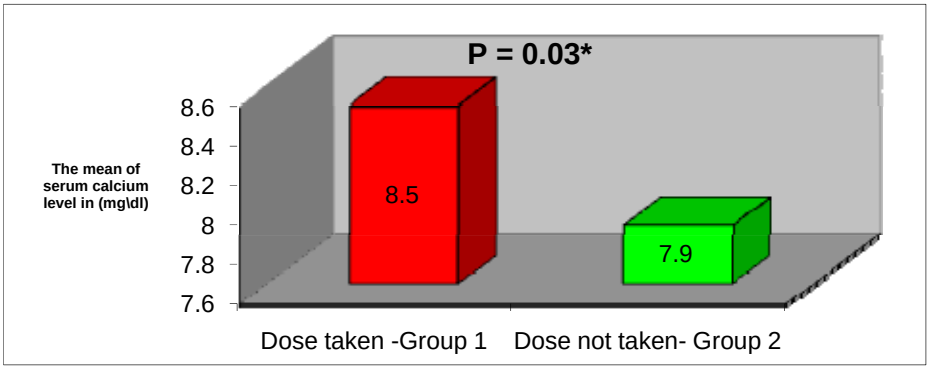

Fig. 3: The mean of serum calcium level in the two groups of the study.

Correlation was done between each of the three parameters; 25 (OH) vitamin D, serum level of calcitonin hormone and serum level of parathyroid hormone versus all other laboratory data of patients included in the study.

There was a significant positive correlation between the serum calcium level and 25 (OH) vitamin D (r = 0.5, P = 0.001), also between serum albumin level and vitamin D (r = 0.4, P = 0.009).

There was no significant correlation between the calcitonin hormone and all of the laboratory data of the patients .

There was a significant negative correlation between the serum level of parathyroid hormone and the calcium level (r = 0.4, P = 0.01), and a negative correlation between the serum level of parathyroid hormone and Magnesium (r = 0.3, P = 0.03) .

Discussion:

Cholestasis can be defined as the accumulation of substances normally excreted in bile e.g. (bilirubin, bile acids, and cholesterol). The process occurs as a result of impaired bile formation by the hepatocytes or from obstruction to the flow of bile through the intra hepatic and extrahepatic biliary tree. (Balistreri, 2002).

As regard the etiological incidence of cholestasis, neonatal hepatitis is considered to be one of the commonest intrahepatic causes of neonatal cholestasis (Hutchin *et al*., 2009). In our study, we had ten patients with neonatal hepatitis representing (25%) of cases, (the commonest etiology).

Also we had nine patients with extrahepatic biliary atresia representing 22.5% of cases which is lower than the results of (Gilmour, *et al.,* 1999**)** who reported 46% of cases with biliary atresia . This is may be attributed to the difference in the population studied and the tools of investigations .

In cholestasis, several factors predispose to vitamin D deficiency. Although 25- hydroxylation in the liver is intact, hepatic secretion of vitamin D binding protein (DBP) is reduced in liver disease leading to lower levels of 25 (OH) vitamin D (Argao *et al.,* 1992). A concentration above 37.5nmol/l is generally considered adequate for those in good health, while levels above 75 nmol/l are proposed to be desirable for achieving normal health, but there is not yet enough evidence to support this (Wang *et al.,* 2010).

In the present study, the mean serum level of vitamin D was 44.7nmol/l in patients and 136.9 nmol/l in controls with a significant p value of 0.005 indicating the presence of vitamin D deficiency especially in patients who were not compliant to vitamin D therapy (n=14,40%), these results are much less than that of (Holick 2007) who estimated the presence of vitamin D deficiency in 92.4% (109 out of 118 patients) with liver diseases and this is can be explained by the fact that his cases were not cholestatic only but different etiologies of liver diseases and were not on vitamin D supplement in contrast to our patients where 60% of them were on regular vitamin D replacement.

In our study, group one (n = 26,65%) had a mean serum level of vitamin D of 51.2nmol/l which is higher than group 2 (n = 14,35%) who had a level of 37.1nmol/l and this can be explained by the difference in compliance. However, both groups had delayed walking as clinical evidence of vitamin D deficiency but there was no broadening or fractures. This agrees with (Klien *et al*., 2002) who stated that hepaticosteodystrophy is multifactorial in origin and factors rather than vitamin D were responsible for its pathogenesis.

Although the etiology of bone disease in biliary atresia remains unclear, vitamin D deficiency has been implicated as a factor in fracture formation (Feranchak *et al*., 2001). In our study, the mean level of 25 OH vitamin D in patients with biliary atresia was 48.2 nmol/l which is reduced (P = 0.02) and this agrees with (Book in 1997) who reported that 66% of infants with cholestasis still have biochemical evidence of vitamin D deficiency despite routine supplementation.

Regarding calcium status, group 2 had a mean serum calcium level of 7.9mg/dl which is slightly lower than group one who had a level of 8.5mg/dl (P = 0.03). There was a positive correlation between serum calcium level and vitamin D (r = 0.5, P = 0.001). These results agree with (Petta *et al.,* 2010) who stated that vitamin D is an important factor for calcium absorption, and its deficiency can lead to decreased calcium absorption which in turn causes bone diseases such as rickets or osteomalacia.

Hypocalcaemia is more evident in progressive familial intrahepatic cholestasis (PFIC) syndrome, we had five patients with PFIC in our study, four of them had lower calcium level (80%), this result is much higher than that of (Nagasaka *et al*., 2004) who reported two out of five patients with PFIC (40%) had episodic hypocalcemia.

Calcitonin is a polypeptide hormone produced primarily by the para follicular cells of the thyroid. It acts to reduce blood calcium opposing the effects of the parathyroid hormone (Costoff, 2008). In bone, calcitonin inhibits calcium resorption by inhibiting the function of mature osteoclasts and by inhibiting the differentiation of osteoclast precursor cells. In kidney, calcitonin inhibits the reabsorption of both calcium and phosphate. In the current study, the total serum level of calcitonin was 7.4 pg/ml in patients and 25.2 pg/ml in controls with p value of 0.006.

In the present study, there was a significant negative correlation between the serum calcium level and parathyroid hormone (r = 0.4, p = 0.01), this result agrees with that of (Poole *et al*., 2005) who stated that low serum calcium concentration results in rapidly increased PTH secretion, also prolonged low serum calcium levels stimulate increased PTH synthesis, and eventually parathyroid hyperplasia..

Conclusion:

Finally, we can conclude that children with cholestatic liver diseases are liable to develop vitamin D and calcium deficiency which can lead to bone complications. Incompliance to treatment especially vitamin D doses was common in 40% of patients which increases the incidence of these complications..

Recommendations:

It is currently recommended that children with chronic cholestasis should have periodic monitoring of serum 25 (OH) vitamin D, calcium, phosphorus and calcitonin hormone. We also recommend proper dose intake (adequate compliance) to minimize the occurrence of vitamin D deficiency.

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