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Stoss therapy versus weekly regimen of vitamin D in children with chronic liver disease: a randomized pilot study

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Abstract

Background Vitamin D, a hormone involved in the regulation of mineral homeostasis, protects skeletal integrity and modulates cell growth and differentiation. Recently, its potential antifibrotic effects have also been identified. Children with chronic liver disease mostly suffer from vitamin D deficiency. However, little knowledge is known regarding the optimum regimen that can be utilized effectively and safely to correct vitamin D deficiency in these patients and whether it could be effective in reversal or at least halting the progressive process of liver fibrosis. This study is conducted to answer these questions.

Results Twenty-four children with chronic liver disease (13 boys and 11 girls) were included in the study. Their age ranged from 4.5 to 11.5 years with median age of 8 years. The aetiology of liver disease was heterogenous with autoimmune hepatitis, glycogen storage disease, or chronic hepatitis, and hepatitis C affects the majority. The patients were divided into two matched groups: group A (n:12) that received stoss parenteral intramuscular vitamin D3 (cholecalciferol) therapy (200,000 IU) once followed by 600 IU/day orally for 6 months (this is equivalent to the RDA as maintenance therapy) and group B (n:12) that received 50,000 IU/week oral vitamin D3 (cholecalciferol) therapy in divided daily doses adding on the maintenance dose 600 IU/day for the first 4 weeks followed by only 600 IU/day orally for the rest of the 6 months (5 months). Following vitamin D3 supplementation, in group A (vitamin D stoss therapy group) and group B (vitamin D oral therapy group), there were statistically significant improvement of Ca, alkaline phosphatase, and vitamin D levels, though there was no difference in between both groups. No significant correlation could be found between vitamin D changes and fibroscan changes in either group.

Conclusion Vitamin D therapy using stoss dose followed by oral therapy or oral vitamin D therapy from the start was equally safe and effective in improving the clinical and laboratory metabolic bone profile abnormalities. Vitamin D effect on liver fibrosis progression or reversion in children is still not understood, and further studies are needed in this field taking in consideration the various causes of liver disease in children.

Keywords Chronic liver disease, Vitamin D deficiency, Fibroscan, Stoss vitamin D therapy, Oral vitamin D therapy

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Background

Vitamin D is a hormone involved in the regulation of mineral homeostasis. It protects skeletal integrity and modulates cell growth and differentiation [1]. It is lipid soluble in nature and interacts with vitamin D receptors and regulates the expression of more than 200 genes, mostly involved in apoptosis, cell growth, and cell differentiation [2].

Vitamin D has been shown to delay hepatic fibrosis by several mechanisms: inhibition of transforming growth factor-beta1 (TGF-β1)-induced stimulation of α-smooth muscle actin expression, decreased expression of collagen I and III, increased expression of several antifibrotic factors such as bone morphogenetic protein 7 (BMP 7) and BMP 8, and inhibition of lipopolysaccharide-mediated activation of hepatic stellate cell [3].

However, it is still not well understood what dose and duration of vitamin D therapy regime could be effective in reversal or at least halting the progressive process of liver fibrosis.

Children with chronic liver disease (CLD) mostly (> 90%) suffer from vitamin D deficiency [4].

Among them, 1/3 have severe vitamin D deficiency. The severity of vitamin D deficiency is directly related to the severity of liver disease [5].

This is attributed to several factors including impaired vitamin D absorption due to portal hypertension enteropathy/cholestasis, decreased vitamin D binding protein production, and impaired hepatic activation stage [4].

Hepatic osteodystrophy is a term used to describe metabolic bone disease in patients with CLD. In children, it affects the growth plate in addition to the existing bone mineral density. Consequently, they are prone to rickets, low bone mass, fractures, and short stature [6].

Therefore, correction of vitamin D deficiency is important to prevent and treat the consequences associated with hepatic osteodystrophy. Little knowledge is known regarding the optimum regimen that can be utilized effectively and safely to correct vitamin D deficiency in these patients [7].

Our study aims to fill this knowledge gap by assessment of two different vitamin D3 (cholecalciferol) regime effects on randomly allocated matched children with chronic liver disease.

Liver biopsy, the gold standard tool to assess hepatic fibrosis, being invasive, is rarely repeated following initial diagnostic biopsy in paediatric cohorts, and there is a real need for noninvasive tools that could assess fibrosis progression or regression. There is a significant positive correlation between fibrosis as assessed in liver biopsy and liver stiffness measurement by fibroscan [8].

Therefore, correlation between vitamin D changes upon vitamin D therapy, and fibroscan changes could be used easily to assess vitamin D antifibrotic potentials. The aim of the study is to assess the efficacy of two different vitamin D regimes in the treatment of vitamin D deficiency in children with CLD. Secondary aims are to study the factors affecting this response (aetiology and severity of CLD utilizing the Child–Pugh classification system) and to evaluate the effect of vitamin D correction on liver fibrosis regression utilizing fibroscan score.

Patients and methods

This study is a prospective double-armed randomized study that was conducted on 24 patients. All subjects were recruited from Hepatology Clinic, Children's Hospital, Ain Shams University. A written consent was taken from the parents/guardians of all participants after the approval of the Ethical Committee of Ain Shams University.

All the children were below the age of 18 years suffering from chronic liver disease were included in the study. Patients with concomitant renal affection, patients who are on anticonvulsant therapy, patients who lost follow-up, patients who refuse to complete at any time throughout the study period, and non-compliant patients for more than 50% of time were excluded from the study.

There were 13 males (54.2%) and 11 females (45.8%), male:female ratio = 1.18%. Their ages ranged from 4.5 to 11.5 years with median age of 8 years. The participants were allocated randomly by alternation into one of two groups. They were followed up for 6 months during the period from April to November 2022. *Group A* (*n*:12) received stoss parenteral intramuscular vitamin D3 (cholecalciferol) therapy (200,000 IU) once followed by 600 IU/day orally for 6 months (this is equivalent to the RDA as maintenance therapy). *Group B* (n:12) received 50,000 IU/week oral vitamin D3 (cholecalciferol) in divided daily doses adding on the maintenance dose 600 IU/day for the first 4 weeks followed by only 600 IU/day orally for the rest of the 6 months (5 months). Both groups received calcium carbonate supplements on 50 mg/kg/day elemental calcium.

All subjects included in the study were subjected to *complete history taking* including the aetiology of the liver disease, any other associated medical conditions, and previous surgery. Symptoms of liver disease (jaundice, abdominal distention, and lower limb oedema) and symptoms of vitamin D deficiency (delayed walking and delayed motor development) were sought. Manifestations of hypervitaminosis D after initiation of the therapy (fatigue, loss of appetite, weight loss, excessive thirst, excessive urination, dehydration, constipation, irritability) and drug history (type, doses, and compliance) were also sought. *Complete history and clinical examination* were done with special emphasis on

anthropometric measures; standard deviation score (SDS) of body mass index (BMI) [9]; abdominal examination; manifestations of liver decompensation (jaundice, ascites, and encephalopathy); manifestations of portal hypertension (hematemesis, melena, splenomegaly, and dilated abdominal veins); and manifestations of vitamin D deficiency (bow legs or knock knees, enlarged wrists and ankles, rickety rosary, and kyphosis). Finally, *investigations* were done as regard laboratory investigations: complete blood count (CBC), liver function tests (serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total and direct serum bilirubin, serum gamma-glutamyl transferase (GGT), international normalized ratio (INR), and serum albumin, serum electrolytes (serum calcium and serum phosphorous), and serum alkaline phosphatase. Radiological investigations including pelviabdominal ultrasound and fibroscan as a noninvasive imaging study for measuring liver fibrosis by transducer probe-induced elastic shear wave that propagates through liver tissue to measure its velocity were done [10]. Fibroscan was done twice before and after vitamin D3 therapy at 0- and 6-month follow-up.

Quantitative estimation of 25(OH)D level was done using enzyme-linked immunoassay (ELISA) at 0- and 6-month follow-up [11].

Statistical analysis

Data were collected, revised, coded, and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations, and ranges when parametric and median, inter-quartile range (IQR) and when data found non-parametric. Then, appropriate statistical analyses were done. The confidence interval was set to 95%, and the margin of error accepted was set to 5%. So, the *P*-value was considered significant as the following: $P > 0.05$: nonsignificant, $P < 0.05$: significant, and $P < 0.01$: highly significant.

Results

Twenty-four children with chronic liver disease (13 males and 11 females) were included in the study. Their age ranged from 4.5 to 11.5 years with median age of 8 years. Demographics and anthropometric measures of the patients are described in Table 1.

The aetiology of chronic liver disease is shown in Table 2. Most of the participant children are compensated (75%) with low percent of complications (25% with manifestations of portal hypertension and 12.5% with vitamin D deficiency features) (Table 2). One-fourth (25%) of the children were on steroids (low dose) (Table 2).

Children in groups A and B were matched regrading age and sex as well as anthropometric measures (Table 3). The aetiology of liver disease, proportions of decompensated/complicated cases, and past medications history were also matched (Table 4). As regard the laboratory results and the imaging findings, both groups were matched (Tables 5 and 6).

Table 1 Age, sex and anthropometrics of the patients

Age, sex and anthropometrics		No. = 24
Age (years)	Median (IQR)	8 (4.5–11.5)
	Range	0.92–16
Sex	Female	11 (45.8%)
	Male	13 (54.2%)
Weight (kg)	Mean ± SD	27.74 ± 13.07
	Range	7.8–48
Weight for height (SDS weight)	Median (IQR)	0.46 (− 0.77–1.11)
	Range	− 2.3–5.82
Height (cm)	Mean ± SD	120.90 ± 27.04
	Range	68.5–158
Height for age (SDS height)	Median (IQR)	− 0.35 (− 1.39–0.28)
	Range	− 2.58–1.5
BMI (kg/m^2)	Mean ± SD	17.87 ± 2.52
	Range	13.4–24.5
BMI SDS	Median (IQR)	0.5 (− 0.25–1.15)
	Range	− 2.6–2.6

Following vitamin D supplementation, in group A (vitamin D stoss therapy group), there was a statistically significant increase of serum Ca level ($P = 0.001$) and statistically significant decrease of serum alkaline phosphatase (ALP) level ($P < 0.001$). While the improvement of PO4 was not statistically significant ($P = 0.293$). Similar patterns appeared in group B with significant increase of serum Ca level and significant decrease of serum ALP with *P*-values at 0.008 and 0.004, respectively, and again the increase of serum PO4 level was again insignificant ($P = 0.507$) (Table 7), comparing both groups together, the difference of serum Ca improvement and alkaline phosphatase level decline was insignificant with *P*-value at 0.977 and 0.707, respectively (Table 8).

Vitamin D levels have improved significantly in group A from mean value of 12.92 ± 3.87 ng/ml (range 5–18 ng/ml) to 30.25 ± 5.58 ng/ml (range 22–38 ng/ml) at the end of follow-up period with *P*-value of < 0.001. Likewise, the improvement of vitamin D levels among group B participants, from mean value of 14.75 ± 3.31 ng/ml (range 7–20 ng/ml) to mean 32.17 ± 4.47 ng/ml (range 25–38 ng/ml), was statistically significant (Table 7). The difference of vitamin D improvement using stoss therapy in comparison with oral therapy was nonsignificant (*P*-value 0.885) (Table 8).

Though the improvement of vitamin D level was satisfactory and reached normal level at the end of the follow-up in both groups, a negative correlation could be

Table 2 Liver disease aetiology, decompensation, complications and drug history of the participants

Aetiology of liver disease		**No**	**%**
Liver disease	Autoimmune hepatitis	6	25.0%
	Glycogen storage disease	6	25.0%
	Chronic hepatitis	3	12.5%
	HCV	3	12.5%
	Wilson's disease	3	12.5%
	Congenital hepatic fibrosis	1	4.2%
	Budd-Chiari disease	1	4.2%
	Niemann-Pick disease	1	4.2%
Decompensation and complications			
Liver decompensation manifestations	Negative	18	75.0%
	Positive	6	25.0%
Portal hypertension	Negative	18	75.0%
	Positive	6	25.0%
Vitamin D deficiency signs	Negative	21	87.5%
	Positive	3	12.5%
Drugs			
Steroids		6	25.0%
Penicillamine		3	12.5%
Azathioprine		2	8.3%
Inderal		6	25%
Marevan		1	4.2%

Table 3 Comparison between group A and B regarding age, sex and anthropometrics

Age, sex and anthropometrics differences		**Group A No. = 12**	**Group B No. = 12**	**Test value**	***p*-value**	**Sig**
Age (years)	Median (IQR)	5.5 (3.75–11.25)	8.5 (6.38–11.5)	−0.926[b]	0.355	NS
	Range	0.92–13	1.5–16			
Sex	Female	5 (41.7%)	6 (50.0%)	0.168[a]	0.682	NS
	Male	7 (58.3%)	6 (50.0%)			
Weight (kg)	Mean ± SD	26.19 ± 13.46	29.29 ± 13.06	−0.573•	0.573	NS
	Range	7.8–47.5	9–48			
Weight for height (SDS weight)	Median (IQR)	0.72 (−0.86–1.11)	0.15 (−0.77–0.97)	−0.462[b]	0.644	NS
	Range	−2.09–5.82	−2.30–2.50			
Height (cm)	Mean ± SD	117.75 ± 27.48	124.04 ± 27.42	−0.561•	0.580	NS
	Range	68.5–157	73.5–158			
Height for age (SDS height)	Median (IQR)	−0.35 (−1.41–0.85)	−0.36 (−1.39 to −0.09)	−0.520[b]	0.603	NS
	Range	−2.58–1.26	−2.50–1.50			
BMI (kg/m^2)	Mean ± SD	17.70 ± 2.61	18.04 ± 2.52	−0.326•	0.748	NS
	Range	13.4–22.2	14.2–24.5			
BMI SDS	Median (IQR)	0.10 (−0.50–1.40)	0.55 (0.35–0.95)	−0.289[b]	0.773	NS
	Range	−2.60–2.60	−2.40–1.70			

[a] Chi-square test

[b] Mann–Whitney test

•Independent t-test

P-value > 0.05, nonsignificant (*NS*); *P*-value < 0.05, significant (*S*); *P*-value < 0.01, highly significant (*HS*)

Table 4 Comparison between groups A and B regarding aetiology of liver disease, liver decompensation, complications and drug medication history

		Group A		Group B		Test value[a]	*p*-value	Sig
		No	%	No	%			
Liver disease	Autoimmune hepatitis	1	8.3%	5	41.7%	9.333	0.230	NS
	Glycogen storage disease	3	25.0%	3	25.0%			
	Chronic hepatitis	1	8.3%	2	16.7%			
	HCV	3	25.0%	0	0.0%			
	Wilson's disease	1	8.3%	2	16.7%			
	Congenital hepatic fibrosis	1	8.3%	0	0.0%			
	Budd Chiari	1	8.3%	0	0.0%			
	Niemann Pick	1	8.3%	0	0.0%			
Liver decompensation symptoms	Negative	9	75.0%	9	75.0%	0.000	1.000	NS
	Positive	3	25.0%	3	25.0%			
Portal hypertension	Negative	8	66.7%	10	83.3%	0.889	0.346	NS
	Positive	4	33.3%	2	16.7%			
Vitamin D deficiency signs	Negative	11	91.7%	10	83.3%	0.381	0.537	NS
	Positive	1	8.3%	2	16.7%			
Steroids		1	8.3%	5	41.7%	3.556	0.059	NS
Penicillamine		1	8.3%	2	16.7%	0.000	1.000	NS
Azathioprine		0	0.0%	2	16.7%	2.182	0.140	NS
Inderal		4	33.3%	2	16.7%	0.889	0.346	NS
Marevan		1	8.3%	0	0.0%	1.043	0.307	NS

P-value > 0.05, nonsignificant (*NS*); *P*-value < 0.05, significant (*S*); *P*-value < 0.01, highly significant (*HS*)

[a] Chi-square test

detected between the age and vitamin D level change especially in group A, and the older the patients, the lower the vitamin D level as in Fig. 1 and Table 9. Correlation between vitamin D levels initially and after vitamin D supplementation in relation to fibroscan changes in groups A and B, described in Tables 10 and 11, shows no significant correlation in both groups. Similarly, no correlation could be detected between vitamin D changes with Child–Pugh scoring system or liver aetiology in either group.

Discussion

Vitamin D deficiency is a common finding in children with chronic liver disease.

In this study, we aimed to compare two regimens of vitamin D3 therapy in children with chronic liver disease by dividing a cohort of patients into two groups that were matched as regards age, sex, aetiology and severity of liver disease as well as BMI, weight and height for age.

Vitamin D3 administration was started in group A as a single IM dose of 200,000 IU, while in group B, it was started with an oral dose of 50,000 IU/week for 4 weeks. Both groups were maintained on the same dose (600 IU orally/day) for 6 months.

The level of vitamin D achieved in both groups was within the normal range with group A mean of 30.25 ± 5.58 ng/ml (range 22–38 ng/ml) and group B mean value of 32.17 ± 4.47 ng/ml (range 25–38 ng/ml). It is suggested that maintenance of serum 25(OH) D level between 30 and 60 ng/mL is ideal, and up to 100 ng/ml is safe [12].

The cumulative dose of vitamin D administered for group A and group B was equal to 308,000 IU. After vitamin D supplementation, serum Ca improvement and serum alkaline phosphatase decline were statistically significant regardless the regime used. Despite the fact that the improvement of calcium level and the decline in alkaline phosphatase were higher in group A than group B, yet it did not reach the level of statistical significance. This could be attributed to the small numbers of children in both groups.

There was no level higher than the risky level of hypervitaminosis D (> 100 ng/ml). So, both regimes were proved to be safe and effective in improvement of vitamin D levels.

The obstacles of vitamin D metabolism in patients with chronic liver disease are at different levels that include defective absorption in patients with portal

Table 5 Comparison between group A and group B regarding laboratory and radiological findings before vitamin D supplementation

		Group A No. = 12	Group B No. = 12	Test value	*p*-value	Sig
TLC (10^3/Ul)	Mean ± SD	6.44 ± 1.67	6.88 ± 2.22	−0.541[b]	0.594	NS
	Range	3.9–10.3	3.7–9.6			
Hb (g/dl)	Mean ± SD	11.07 ± 1.05	11.20 ± 1.57	−0.245[b]	0.809	NS
	Range	8.4–12.6	8.2–13.6			
PLTs (10^3/Ul)	Median (IQR)	268 (169.5–403)	279.5 (189.5–311)	−0.231[c]	0.817	NS
	Range	83–484	61–523			
ALT (IU/l)	Median (IQR)	63 (27–231)	39.5 (28.5–53)	−1.184[c]	0.236	NS
	Range	14–642	14–398			
AST (IU/l)	Median (IQR)	62.5 (43.5–348.5)	48.5 (42–77)	−0.635[c]	0.525	NS
	Range	20–676	29–919			
Gama GT	Median (IQR)	55.5 (39–70)	89 (51–122)	−1.530[c]	0.126	NS
	Range	24–131	34–170			
Total bilirubin (mg/dl)	Median (IQR)	0.85 (0.65–1.4)	0.75 (0.5–1.15)	−0.521[c]	0.603	NS
	Range	0.2–2.4	0.3–2.4			
Direct bilirubin (mg/dl)	Median (IQR)	0.25 (0.11–0.55)	0.15 (0.1–0.3)	−1.013[c]	0.311	NS
	Range	0.1–0.8	0.1–1.6			
Albumin (g/dl)	Mean ± SD	3.95 ± 0.50	4.03 ± 0.42	−0.398	0.695	NS
	Range	3.1–4.5	3.3–4.6			
INR	Mean ± SD	1.26 ± 0.40	1.17 ± 0.18	0.774	0.447	NS
	Range	0.9–1.9	1–1.54			
Child–Pugh score	Median (IQR)	6.00 (6.00–8.50)	6.00 (5.50–7.50)	−0.991[c]	0.322	NS
	Range	5.00–13.00	5.00–13.00			
Child–Pugh score	Class A	7 (58.3%)	9 (75.0%)	0.783[a]	0.676	NS
	Class B	3 (25.0%)	2 (16.7%)			
	Class C	2 (16.7%)	1 (8.3%)			
PA ultrasound	Normal	2 (16.7%)	2 (16.7%)	2.000[a]	0.572	NS
	Hepatosplenomegaly	6 (50.0%)	3 (25.0%)			
	Hepatomegaly	3 (25.0%)	6 (50.0%)			
	Cirrhotic liver	1 (8.3%)	1 (8.3%)			
Fibroscan (kPa)	Median (IQR)	6.45(5.85–8.2)	6.05(4.8–8.2)	−0.492[c]	0.623	NS
	Range	3.3–17.9	4–20.6			
	F0	6 (50.0%)	6 (50.0%)	3.200[a]	0.783	NS
	F1	0 (0.0%)	1 (8.3%)			
	F2	2 (16.6%)	1 (8.3%)			
	F3	3 (25.0%)	3 (25%)			
	F4	1 (8.3%)	1 (8.3%)			

P-value > 0.05, nonsignificant (*NS*); *P*-value < 0.05, significant (*S*); *P*-value < 0.01, highly significant (*HS*)

[a] Chi-square test

[b] Independent *t*-test

[c] Mann–Whitney test

hypertension, defective binding proteins production by the liver, and defective vitamin D activation in the diseased liver.

Maintenance dose ensured maintained vitamin D bioavailability based on the fact that the half-life of vitamin D level is 28 days, and to achieve plateau level, it needs vitamin D administration for 1–2 months [13].

Therefore, stoss therapy without maintenance doses would result in wasting of the vitamin D available by a single dose. Lal et al. in their study proved improvement of vitamin D level with regular oral vitamin D intake in comparison with the restricted stoss therapy use [7].

Despite the presence of vitamin D deficiency in the participants, clinical manifestations of vitamin D

Table 6 Comparison between group A and group B regarding metabolic profile and vitamin D before vitamin D supplementation

At the beginning	Group A		Group B	Test value[a]	*p*-value	Sig
	No. = 12		No. = 12			
Serum Ca (mg/dl)	Mean ± SD	9.04 ± 1.01	9.12 ± 0.86	− 0.196	0.846	NS
	Range	7.3–10.4	7.4–10.3			
Serum Po_4 (mg/dl)	Mean ± SD	4.63 ± 1.04	4.25 ± 0.88	0.976	0.340	NS
	Range	2.9–6.2	3.1–5.8			
Serum ALP (U/l)	Mean ± SD	287.75 ± 66.98	287.08 ± 46.38	0.028	0.978	NS
	Range	190–421	197–361			
25(OH) vitamin D (ng/ml)	Mean ± SD	12.92 ± 3.87	14.75 ± 3.31	− 1.247	0.225	NS
	Range	5–18	7–20			

P-value > 0.05, nonsignificant (*NS*); *P*-value < 0.05, significant (*S*); *P*-value < 0.01, highly significant (*HS*). *P*-value > 0.05, nonsignificant (*NS*); *P*-value < 0.05, significant (*S*); *P*-value < 0.01, highly significant (*HS*)

[a] Independent *t*-test

Table 7 Metabolic profile changes and fibroscan after vitamin D supplementation in both groups

Group A		At the beginning	At the end	Test value	*p*-value	Sig
		No. = 12	No. = 12			
S. Ca (mg/dl)	Mean ± SD	9.04 ± 1.01	9.79 ± 0.75	4.210•	0.001	HS
	Range	7.3–10.4	8.5–11			
S. Po_4 (mg/dl)	Mean ± SD	4.63 ± 1.04	4.96 ± 0.76	1.105•	0.293	NS
	Range	2.9–6.2	3.7–6.2			
S. ALP (U/l)	Mean ± SD	287.75 ± 66.98	205.00 ± 36.56	− 6.224•	0.00	HS
	Range	190–421	163–272			
Vitamin D (ng/ml)	Mean ± SD	12.92 ± 3.87	30.25 ± 5.58	31.056•	0.00	HS
	Range	5–18	22–38			
Fibroscan (kPa)	Median (IQR)	6.45 (5.85–8.2)	6.55 (5.1–8)	0.393•	0.694	NS
	Range	3.3–17.9	3.5–23.9			
	F0	6 (50.0%)	5 (41.7%)	4.291*	0.637	NS
	F1	0 (0.0%)	2 (16.7%)			
	F2	2 (16.6%)	1 (8.3%)			
	F3	3 (25.0%)	3 (25.0%)			
	F4	1 (8.3%)	1 (8.3%)			
Group B		**At the beginning**	**At the end**	**Test value**	***p*-value**	**Sig**
		No. = 12	**No. = 12**			
S. Ca (mg/dl)	Mean ± SD	9.12 ± 0.86	9.94 ± 0.60	3.217•	0.008	HS
	Range	7.4–10.3	8.9–10.9			
S. Po_4 (mg/dl)	Mean ± SD	4.25 ± 0.88	4.38 ± 0.62	0.686•	0.507	NS
	Range	3.1–5.8	3–5.1			
S. ALP (U/l)	Mean ± SD	287.08 ± 46.38	196.58 ± 71.14	− 3.584•	0.004	HS
	Range	197–361	106–310			
Vitamin D (ng/ml)	Mean ± SD	14.75 ± 3.31	32.17 ± 4.47	16.978•	0.000	HS
	Range	7–20	25–38			
Fibroscan (kPa)	Median (IQR)	6.05 (4.8–8.2)	5.85 (5.1–8)	− 0.178‡	0.859	NS
	Range	4–20.6	420			
	F0	6 (50.0%)	6 (50.0%)	1.333*	0.931	NS
	F1	1 (8.3%)	1 (8.3%)			
	F2	2 (16.6%)	2 (16.7%)			
	F3	3 (25.0%)	2 (16.7%)			
	F4	1 (8.3%)	1 (8.3%)			

•paired t test

*Chi-square test

‡Wilcoxon test

Table 8 Comparing metabolic profile, vitamin D level and fibroscan changes after vitamin D supplementation between both groups

Change		Group A No. = 12	Group B No. = 12	Test value‡	*p*-value	Sig
S. Ca (mg/dl)	Mean ± SD	0.75 ± 0.62	0.83 ± 0.89	− 0.029	0.977	NS
	Range	− 0.6–1.7	− 0.6–2.3			
S. Po_4 (mg/dl)	Mean ± SD	0.32 ± 1.02	0.13 ± 0.63	− 0.491	0.623	NS
	Range	− 1.5–2.5	− 0.9–1			
S. ALP (U/l)	Mean ± SD	− 82.75 ± 46.06	− 90.50 ± 87.48	− 0.375	0.707	NS
	Range	− 162 to − 9	− 217–33			
Vitamin D (ng/ml)	Mean ± SD	17.33 ± 4.60	17.42 ± 3.55	− 0.145	0.885	NS
	Range	9–25	10–22			
Fibroscan	Mean ± SD	0.21 ± 2.00	0.27 ± 2.03	− 0.289	0.772	NS
	Range	− 1.9–6	− 3–5.9			

‡Mann Whitnet test

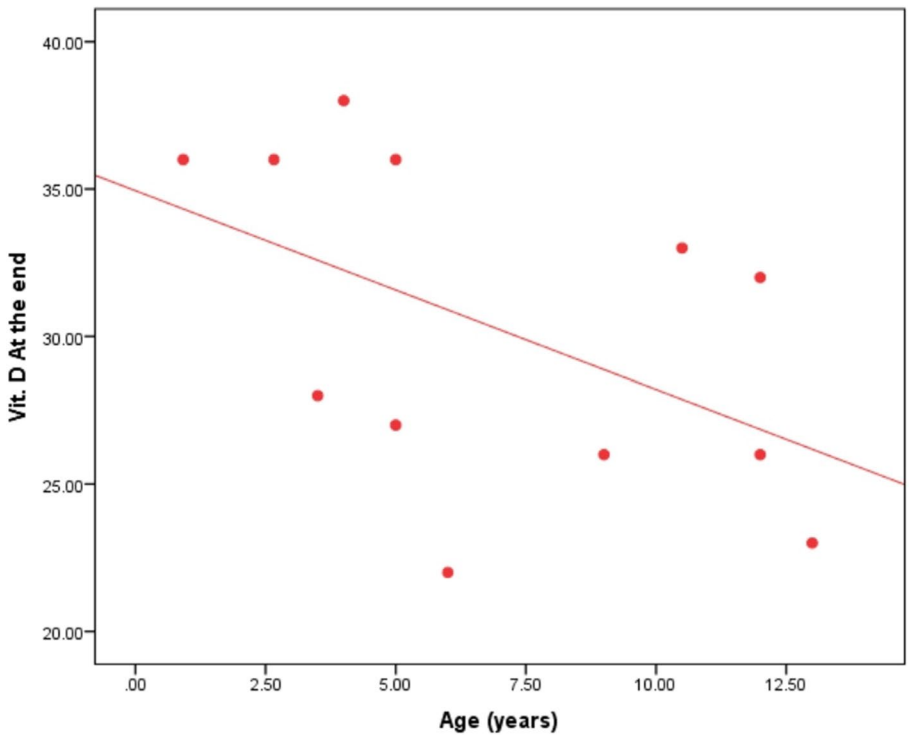

Fig. 1 A negative correlation relation between patient's age and the level of vitamin D difference in group A

deficiency were present in only three patients (12.5%), highlighting the importance of routine assessment of vitamin D level in children with chronic liver disease and hence treating them, not relying only on the overt clinical appearance of hypovitaminosis. This observation has been noticed also in a study which identified that discrepancy between the clinical presentation and the laboratory findings as only 25.4% of patients with CLD who had vitamin D deficiency developed rickets [7].

There was a negative correlation detected between the child's age and vitamin D level reached at the end of therapy especially in group A (although all vitamin D level at the end was within the normal range). This may indicate that vitamin D dose tailoring based on age and weight might be needed. Further studies are required to validate/ disprove this.

It has been observed in adults' studies that vitamin D therapy resulted in improvement of fibrosis. Calcitriol showed anti-fibrotic effects in lung fibroblasts in vitro

Table 9 Correlation between vitamin D level changes after supplementation in relation to anthropometric measures, metabolic profile and fibroscan

	Vit. D change			
	Group A		Group B	
	R	*p*-value	*r*	*p*-value
Age (years)	**−0.616***	**0.033**	−0.046	0.887
Weight (kg)	−0.453	0.139	0.049	0.879
Height (cm)	−0.408	0.188	0.063	0.845
BMI (kg/m^2)	−0.271	0.395	−0.324	0.304
S. Ca (mg/dl)	**0.635***	**0.027**	−0.099	0.759
S. Po_4 (mg/dl)	0.023	0.944	−0.039	0.905
S. ALP (U/l)	−0.109	0.736	0.203	0.526
ALT (IU/l)	0.000	1.000	0.346	0.271
AST (IU/l)	−0.295	0.351	0.423	0.170
Albumin (g/dl)	0.021	0.948	−0.094	0.772
INR	−0.004	0.991	−0.416	0.179
Fibroscan	0.381	0.222	0.512	0.089
Child–Pugh score	−0.180	0.575	−0.179	0.578
Wt for height (SDS wt.)	−0.337	0.283	−0.021	0.948
Height for age (SDS ht.)	−0.109	0.736	0.346	0.271
BMI SDS	−0.295	0.351	0.034	0.917

*significant correlation

[14, 15], as well as both in vitro and in vivo rat models of liver fibrosis. It is suggested that 1α,25(OH)$_2$D suppresses hepatic stellate cells proliferation, and expression of cyclin D1, tissue inhibitor of metalloproteinase 1, and collagen Iα1 in vitro. In vivo, 1α,25(OH)$_2$D decreases α-SMA expression and collagen levels and prevents the development of cirrhosis by thioacetamide (TAA) [16].

Vitamin D level > 50 nmol/l may have been suggested to have a role in decreasing the frequency of rapid fibrosis progression in chronic hepatitis C [17].

However, the clinical importance of vitamin D as an antifibrotic agent remains to be determined: whether to prevent the progression of fibrosis or to reverse the fibrosis. In addition, factors such as liver disease aetiology, age, severity of liver fibrosis, vitamin D dose, and treatment durations may play a role determining who would benefit from the antifibrotic effect of vitamin D.

This study is not without limitations; the heterogeneity of disease aetiology and liver fibrosis severity are limitations to assess the efficacy of vitamin D therapy on liver fibrosis. It is a single-centre study on 24 children; multicentric study on larger population with more homogenous groups is required to validate the findings. The effect of sun exposure was not studied, and does it share in improvement of vitamin D level or not. Similarly, daily physical activities/dietary habits have not not be recorded.

In this study, there was no significant fibroscan changes detected after vitamin D therapy in either group, and, likewise, the difference of changes between both groups was not significant. This was also observed by El Amrousy et al. after assessment of efficacy of vitamin D therapy in 109 children with non-alcoholic steatohepatitis (NASH). Despite improvements detected in fibroscan at the end of therapy, it was not statistically significant ($P = 0.986$) [18].

Conclusion

Vitamin D therapy using stoss therapy or oral vitamin D therapy was equally safe and effective in improving the clinical and laboratory metabolic bone profile

Table 10 Correlation between vitamin D levels with fibroscan grade at the beginning and at the end in group A

Fibro-scan grade		Group A		Test value[a]	*p*-value	Sig
		Vit. D				
		Mean ± SD	Range			
At the beginning	F0	13.33 ± 3.33	9–18	0.842	0.540	NS
	F1	-	-			
	F2	16.00 ± 0.00	16–16			
	F3	11.00 ± 5.29	5–15			
	F4	9.00 ± 0.00	9–9			
At the end	F0	32.60 ± 5.64	26–38	1.036	0.474	NS
	F1	29.50 ± 4.95	26–33			
	F2	22.00 ± 0.00	22–22			
	F3	32.00 ± 5.66	28–36			
	F4	23.00 ± 0.00	23–23			

P-value > 0.05, nonsignificant (*NS*); *P*-value < 0.05, significant (*S*); *P*-value < 0.01, highly significant (HS)

[a] One-way ANOVA test

Table 11 Relation between vitamin D with fibroscan grade at the beginning and at the end in group B

Fibroscan grade		Group B		Test value[a]	*p*-value	Sig
		Vit. D				
		Mean ± SD	Range			
At the beginning	F0	13.83 ± 3.54	7–16	0.452	0.799	NS
	F1	15.00 ± 0.00	15–15			
	F2	14.00 ± 0.00	14–14			
	F3	16.50 ± 4.95	13–20			
	F4	13.00 ± 0.00	13–13			
At the end	F0	31.83 ± 4.96	25–38	0.824	0.549	NS
	F1	35.00 ± 0.00	35–35			
	F2	34.00 ± 1.41	33–35			
	F3	33.50 ± 4.95	30–37			
	F4	25.00 ± 0.00	25–25			

P-value > 0.05, nonsignificant (*NS*); *P*-value < 0.05, significant (*S*); *P*-value < 0.01, highly significant (*HS*)

[a] One-way ANOVA test

abnormalities. Vitamin D effect on liver fibrosis progression or reversal in children is still not understood, and further studies are needed in this field taking in consideration the various causes of liver disease in children.

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
BMP	Bone morphogenetic protein
CLD	Chronic liver disease
ELISA	Enzyme-linked immunoassay
CBC	Complete blood count
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
INR	International normalized ratio
IQR	Interquartile range
NASH	Non-alcoholic steatohepatitis
PA sonar	Pelviabdominal sonar
PLTs	Platelets
SDS	Standard deviation score
TAA	Thioacetamide
TGF-β1	Transforming growth factor-beta1
TLC	Total leucocytic count

Authors' contributions

HSMA, collected data, designed the study and wrote the manuscript. LBE, designed the study, analysed data and reviewed the manuscript critically. NNT, SMEM, GGN and MAN analysed data and reviewed the manuscript critically. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This is a prospective, double-armed randomized study that was conducted on human participants with the approval of the Human Research Ethical Committee from Ain Shams University. Consents have been taken from the participants' guardians with no obligations and had the right to leave the study whenever they want.

Consent for publication

Consents taken from the participants' guardians also involve their acceptance for sharing data anonymously in research.

Competing interests

The authors declare that they have no competing interests.

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