Original article

Pegylated interferon α/ribavirin therapy enhances bone mineral density in children with chronic genotype 4 HCV infection

Ahmed Megahed, Nanees Salem, Abeer Fathy, Tarik Barakat, Mona Abd El Latif Alsayed, Suzy Abd El Mabood, Khaled R Zalata, Ahmed F Abdalla

Mansoura, Egypt

Background: **The impact of chronic hepatitis C (CHC) on bone mineral density (BMD) has been well studied in adults with a relative paucity of data in children, especially concerning effect of treatment with pegylated interferon (PEG-IFN) plus ribavirin (RV). In the current work, we assessed prospectively changes in BMD in children with CHC before, during, and after treatment.**

Methods: **Forty-six consecutive children with non-cirrhotic genotype 4 CHC were subjected to dual-energy X-ray absorptiometry at baseline, 24 weeks, 48 weeks of therapy and 24 weeks after treatment. BMD, bone mineral content (BMC), and *Z* score of lumbar spine (L2-L4) were reported. Tanner pubertal stage, viral load, liver function tests, serum calcium, phosphorus, alkaline phosphatase, parathyroid hormone, and liver histopathology were assessed in all included children.**

Results: **Thirty (65.2%) patients had normal BMD, 10 (21.7%) were at risk for low BMD, and 6 (13.1%) had low BMD for chronological age. Patients with low BMD were significantly older (*P*=0.001), with higher frequency of delayed puberty than other groups (*P*=0.002). Baseline densitometric parameters (BMD & BMC) were significantly positively correlated with patients' age, weight, height, body mass index and hemoglobin level; while they were insignificantly correlated with basal viral load, histopathology activity index and fibrosis score. Densitometric parameters improved significantly on PEG-IFN plus RV treatment, this improvement was found to be sustainable 24 weeks after therapy.**

Conclusions: **Low BMD is detectable in a proportion of CHC children. Antiviral therapy leads to a sustainable increase in BMD.**

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Key words: **bone mineral density; chronic hepatitis C; pegylated interferon; ribavirin**

Introduction

Chronic hepatitis C (CHC) continues to be a global health problem; it has high mortality, morbidity and economic impact. Currently, 3% of the world population are chronically infected with hepatitis C.[1,2] Egypt has the highest hepatitis C virus (HCV) prevalence worldwide, estimated to be 14.7% among adult population.[3,4] Prevalence of HCV infection in children is less clear;[5] the prevalence rates in Egypt were low in the 1990s among non-transfusion dependent children,[6] however another series reported prevalence rate to be 2% among children.[7]

Compared with adults, children with CHC infection usually have a mild and slowly progressive disease that unlikely progresses to cirrhosis.[8] Pegylated interferon alpha (PEG-IFNα) with ribavirin (RV) is still the treatment of choice for CHC in children as young as 3 years of age.[9] Direct-acting antivirals (DAAs) drugs have been approved for the treatment of CHC in adult population;[10] however, their usage in children is not approved yet. Different clinical trials are now running for evaluation of DAAs in pediatric HCV population.[11,12]

The term "hepatic osteodystrophy" defines the metabolic bone disorders occurring in individuals with chronic liver disease;[13-15] it commonly affects patients with

Author Affiliations: Pediatric Gastroenterology and Hepatology Unit (Megahed A, Fathy A, Barakat T, Alsayed MAEL, Abdalla AF), Pediatric Endocrinology Unit (Salem N), Pediatric Hematology & Oncology Unit (Mabood SAE), Mansoura University Children's Hospital, Faculty of Medicine, Mansoura University, Egypt; Pathology Department, Faculty of Medicine, Mansoura University, Egypt (Zalata KR)

Corresponding Author: Abeer Fathy, MD, Pediatric Gastroenterology and Hepatology Unit, Mansoura University Children's Hospital, Al Gomhoria Street, Mansoura, Egypt (Tel: +2 01224642996; Fax: +2 0502220679; Email: abeerfathy2000@yahoo.com)

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CHC who had severe cholestasis or liver cirrhosis.[14-16] The participation of vitamin D-parathyroid hormone (PTH) axis disturbance in the bone loss in these patients is controversial, some studies in adults reported unclear role[14,17] while others reported its relevance.[18]

Treatment of CHC with INF plus RV may induce bone loss with RV-dependent changes related to bone mineral metabolism,[19] though other reports showed improvement in bone mineral density (BMD) with INF plus RV therapy.[20] No reports discussed the effect of DAAs on BMD, thus it should be evaluated in future studies.

Despite the significant number of children infected with HCV, there is a relative paucity of data regarding the impact of CHC on bone homeostasis.[21-23] Furthermore, the influence of PEG-IFN α plus RV on bone metabolism in children has been characterized only in few reports.[24] The potential benefit of sustained eradication of HCV on BMD is unknown, and to the best of our knowledge, there are no previous reports on BMD in children with genotype 4 CHC. Therefore, we prospectively investigated lumbar spine BMD in 46 children with CHC genotype 4 infection treated by PEG-IFN α plus RV at baseline, during therapy and 24 weeks after end of the treatment.

Methods

Patients

Forty-six consecutive patients with CHC genotype 4 presented at Hepatology Outpatient Clinic, Mansoura University Children's Hospital, Egypt between January 2011 and January 2013 were enrolled in the present prospective study. The principles outlined in the Declaration of Helsinki were followed and informed consents were obtained after study protocol approval by the local ethical committee in the Faculty of medicine, Mansoura University, Egypt.

All included patients had evidence of CHC infection (positive anti-HCV antibody for more than 6 months and positive HCV-RNA by PCR). Histological examination of liver biopsy was done for all patients. A single expert pathologist reported fibrosis and necroinflammatory injury according to the modified Knodell score by Ishak, in which inflammatory activity is graded from 0 to 18 and fibrosis is graded from 0 to 6.[25]

Exclusion criteria

None of included children had cirrhosis or decompensated liver disease; obesity [body mass index (BMI) >95th percentile]; endorinologic disorders affecting bone metabolism (thyroid, parathyroid diseases, Cushing's disease) or any contraindication for PEG-IFN α plus RV treatment. None of the patients received vitamin D, calcium or any other osteoporosis-specific pharmacological therapy prior to or during the study period. All patient with previous diagnosis of leukemia or solid tumor enrolled in this study had finished their chemotherapy courses at least 12 months before enrollment. Other causes of liver diseases either infectious or metabolic were excluded.

Examination

All included children were subjected to thorough history and careful physical examination including detailed anthropometric measurements and pubertal staging. Laboratory work-up included the following: serum bilirubin (total and direct), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), albumin, alkaline phosphatase (ALP), calcium, phosphorus, and PTH. Liver biopsies were taken as a part of the patient's initial evaluation.

Pubertal assessment

Each patient was examined to assess the pubertal stage according to Tanner. Delayed puberty was defined as the absence of any breast development at the age of 13 years in girls or the absence of increase in testicular volume (<4 mL) at the age of 14 years in boys.[26] Arrested puberty was definded as failure of puberty to progress with a lag in normal pubertal maturation or some pubertal aspects regression (e.g., shrunken breasts or softening testicles), also it included those who needed more than 5 years to complete pubertal development.[27]

Treatment protocol and patients derivation

All patients received combined PEG-IFN α 2b and RV according to the published guidelines.[28] Patients were monitored for drug side effects, particularly thyroid and hematologic disorders, with possible dose modification when needed. None of our patients had developed drug side effect necessitating stopping of treatment.

Viral load was assessed using quantitative RT-PCR at completed 12th, 24th, 48th weeks of therapy, and 24 weeks after treatment for sustained virologic response (SVR). Derivation and definitions of the study population are shown in Fig..

Bone densitometry measurements

Bone densitometry at the postero-anterior lumbar spine (L2-L4) was performed using dual energy X-ray absorptiometry (DXA) (Lunar, DPX IQ-USA, software version 4.5). Sequential measurements were performed at baseline prior to antiviral treatment (46/46 patients), after 24 weeks of antiviral therapy (29/46 patients), after 48 weeks of antiviral therapy (25/46 patients) and at the end of a 24-week follow-up (off-therapy) period (week-72) (25/46 patients).

DXA scanning is the preferred method for measurement of bone density in children;[29] the recommendation of International Society for Clinical Densitometry with a minimum monitoring time interval of 6 months was considered.[30]

The same well-trained technician performed all scans and a single observer performed all analyses. Densitometric data were reported as bone mineral content (BMC, in g) referred to the quantity of bone mineral within the scanned area. BMD was derived by dividing the BMC by the scanned bone area (g/cm^2). BMD *Z* score was calculated based on age- and gender-specific normative reference data for BMD in Egyptian children obtained from 352 control children and adolescents.[31] Considering the similarity of geographical location, genetic background, nutritional status and daily lifestyle, the densometric data obtained from this large group of healthy Egyptian children and adolescents were considered as useful references to assess BMD status in our study.

The DXA results were interpreted using the preferred descriptive terminology in childhood as follow: "at risk for low BMD for chronologic age" when BMD *Z* score is between -1.0 and -1.9, and "low BMD for chronologic age" when BMD *z*-score is less than or equal to -2.0.[32]

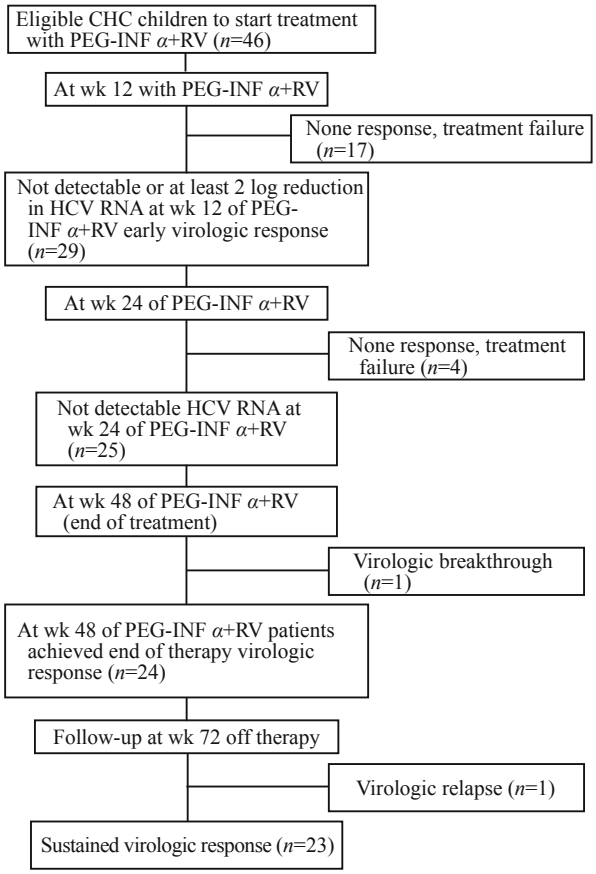

Fig. Derivation and definitions of the study population. CHC: chronic hepatitis C; PEG-INF: pegylated interferon; RV: ribavirin; HCV: hepatitis C virus.

Statistical analysis

Statistical analysis was performed using SPSS 16 software for Windows (SPSS Inc, Chicago, Ill), setting statistical significance at $P<0.05$. Baseline characteristics were described using mean±standard deviation for continuous data and frequency (*n*%) for categorical data.

The Mann-Whitney *U*, the paired-sample *t* and ANOVA tests were applied for comparing continuous variables, while the Chi-squared or Fisher's exact probability test were applied for categorical data. Correlations were done using Spearman's correlation. Multiple regression analysis of various clinical, laboratory and pathologic parameters was also performed.

Results

Forty six children (37 males and 9 females; mean age 10.47±3.95 years) with CHC genotype 4 infection were enrolled; all were non-cirrhotics. Basal laboratory results were as follows: ALT (52.02±42.12 U/L), AST (46.05±34.43 U/L), GGT (41.86±45.58 U/L), total bilirubin (0.68±0.2 mg/dL), albumin (4.59±1.9 g/dL),

Table 1. Baseline clinicopathological characteristics of studied CHC children (*n*=46)

Parameters	Results
Age in y, mean±SD (range)	10.47±3.95 (4-18)
Gender (male/female), *n* (%)	37 (80.4)/9 (19.6)
Height *Z* score, mean±SD	-0.36±0.87
Weight *Z* score, mean±SD	-0.55±0.74
BMI *Z* score, mean±SD	-0.46±0.81
Probable duration of disease in y, mean±SD	5.85±3.24
Probable mode of acquisition, *n* (%)	
Transfusion	29 (63.0)
Intra-familial (other HCV positive family member)	10 (21.7)
Others	7 (15.3)
Co-morbidities, *n* (%)	
No co-morbidities	16 (34.8)
Past co-morbidities	30 (65.2)
Leukemia	9 (19.6)
Solid tumor	12 (26.1)
Renal problem (operated VUR)	4 (8.7)
Chronic anemia	3 (6.5)
Congenital heart diseases	2 (4.3)
Histology activity index, *n* (%)	
None	0 (0)
Minimal (1-3)	12 (26.1)
Mild (4-6)	24 (52.2)
Moderate (7-9)	10 (21.7)
Marked (10-18)	0 (0)
Fibrosis score, *n* (%)	
No & portal-periportal fibrosis (Ishak 0-2)	31 (67.4)
Bridging fibrosis (Ishak 3-4)	15 (32.6)
Advanced fibrosis & cirrhosis (Ishak 5-6)	0 (0)

CHC: chronic hepatitis C; HCV: hepatitis C virus; VUR: vesicoureteric reflux; SD: standard deviation.

prothrombin time (13.6±3.18 seconds), HCV RNA (1.5±2.4 IU/mL×10^6), serum calcium (9.06±0.65 mg/dL), phosphorus (5.64±0.55 mg/dL), ALP (265.5±137.45 U/L) and PTH (30.13±8.08 mg/dL). Clincoepidemiologic data and liver histopathology results are shown in Table 1.

Baseline densitometry data of studied children with CHC

Baseline DXA scan of lumbar spine revealed BMD mean value (0.731±0.17; range between 0.46 and 1.17); BMC (19.48±11.57; range between 3.75 and 52.21) and BMD *Z* score (-0.614±1.097, range between -3.50 and 1.31). All patients had normal laboratory results for serum calcium, phosphorus and PTH.

Among the 46 included CHC children, 30 (65.2%) patients were of normal BMD, 10 (21.7%) were at risk for low BMD, and 6 (13.1%) had low BMD for chronological age. Comparative analysis of different clincoepidemiologic, laboratory and pathologic parameters among these groups is shown in Table 2.

Patients with low BMD were significantly older as compared with other groups (P=0.001). A statistically significant higher frequency of delayed puberty (50%) was noticed among these patients. Regression analysis failed to demonstrate any independent parameter that can distinguish patients with low BMD.

All HCV children with low BMD, a part from one patient with normal puberty, had past medical problems (acute lymphoblastic leukemia in 2 patients; non-Hodgkin lymphoma in 1 patient; 1 patient with operated posterior urethral valve and recurrent urinary tract infection; and another patient with chronic iron

Table 2. Comparative analysis of different baseline parameters among patients' densitometric groups (*n*=46)

Parameters	Normal BMD (*n*=30)	At risk for low BMD (*n*=10)	Low BMD (*n*=6)	*P* value
Age in y, mean±SD	9.0±3.2	12.3±4.4	14.8±1.4	0.001
Sex (M/F), *n*	23/7	9/1	5/1	0.615
Probable duration of illness in y	5.4±2.9	6.6±2.8	6.8±5.2	0.501
Weight *Z* score, mean±SD (range)	-0.48±0.61 (-1.80-0.48)	-0.62±0.75 (-1.41-1.08)	-0.82±1.27 (-2.0-1.23)	0.573
Height *Z* score, mean±SD (range)	-0.32±0.76 (-1.57-1.59)	-0.42±1.04 (-1.85-1.62)	-0.44±1.22 (-1.55-1.80)	0.919
BMI *Z* score, mean±SD (range)	-0.45±0.71 (-1.90-0.99)	-0.44±0.92 (-1.45-1.75)	-0.54±1.20 (-2.08-1.06)	0.964
Pubertal stage, *n* (%)				
Pre-pubertal	24 (80.0)	4 (40)	1 (16.7)	
Arrested puberty	-	1 (10)	1 (16.7)	0.002
Normal puberty	5 (16.7)	2 (20)	1 (16.7)	
Delayed puberty	1 (3.3)	3 (30)	3 (50.0)	
Viral load (IU/mL×10^6), mean±SD	1.40±1.90	2.50±3.70	7.30±1.40	0.316
Serum calcium (mg/dL), mean±SD	9.09±0.60	8.98±0.79	9.07±0.81	0.910
Serum phosphate (mg/dL), mean±SD	5.61±0.51	5.73±0.62	5.62±0.72	0.841
Serum total ALK phospahtase (U/L), mean±SD	281.43±143.17	228.50±119.45	247.50±144.26	0.551
Serum parathyroid hormone (mg/dL), mean±SD	29.65±7.44	31.75±11.06	29.87±6.27	0.780
Past co-morbidities, *n* (%)				
Absent	12 (40.0)	3 (30)	1 (16.7)	0.440
Present	18 (60.0)	7 (70)	5 (83.3)	
Histology activity index, *n* (%)				
Minimal (1-3)	9 (30.0)	2 (20)	1 (16.7)	
Mild (4-6)	16 (53.3)	5 (50)	3 (50.0)	0.815
Moderate (7-9)	5 (16.7)	3 (30)	2 (33.3)	
Histology fibrosis score, *n* (%)				
No and portal-periportal fibrosis (Ishak 0-2)	21 (70.0)	6 (60)	4 (66.7)	0.845
Bridging fibrosis (Ishak 3-4)	9 (30.0)	4 (40)	2 (33.3)	

SD: standard deviation; BMD: bone mineral density; M: male; F: female; BMI: body mass index; ALK: anaplastic lymphoma kinase.

Table 3. Changes in densitometric data during and after PEG-IFN α plus RV therapy

Variables	Baseline densitometric data		Densitometric data 24th wk of therapy (*n*=29)		Densitometric data 48th wk of therapy (*n*=25)			Densitometric data 72nd wk (off-therapy) (*n*=25)		
	Patients completed 24 wk therapy (*n*=29)	Patients completed 48 wk therapy (*n*=25)	Measurements	P^*	Measurements	P^*	$P^\dagger$	Measurements	P^*	$P^\ddagger$
BMD, mean±SD	0.74±0.17	0.75±0.18	0.80±0.17	<0.001	0.84±0.18	<0.001	0.003	0.82±0.20	<0.001	0.121
BMC, mean±SD	20.32±12.48	21.37±13.14	22.57±12.60	<0.001	25.85±13.49	<0.001	<0.001	25.21±13.96	<0.001	0.104
Z score, median (range)	-0.51 (-3.50-1.31)	-0.66 (-3.50-1.31)	0.10 (-2.40-2.07)	<0.001	0.12 (-3.0-2.24)	<0.001	0.655	0.11 (-3.4-2.3)	0.461	0.080

*: test of significance for densitometric data changes from baseline; †: test of significance for densitometric data changes at 48th wk of therapy *vs.* 24th wk of therapy; ‡: test of significance for densitometric data changes at 72nd wk (off-therapy) therapy *vs*. 48th wk of therapy. SD: standard deviation; BMD: bone mineral density; BMC: bone mineral content.

deficiency anemia). The average duration for these past medical coditions was 4±1.2 years; all patients had improved these conditions prior to receiving antiviral therapy.

Baseline densitometric parameters (BMD & BMC) were significantly positively correlated with patients' age, weight, height, BMI and hemoglobin level (r=0.79, 0.81, 0.8, 0.61 and 0.48, respectively; P<0.001) for the former; (r=0.81, 0.85, 0.82, 0.64 and 0.51, respectively; P<0.001) for the later. While they were insignificantly correlated with the probable duration of the disease, ALT, AST, GGT, total bilirubin, direct bilirubin, albumin levels, basal viral load, histopathology activity index and fibrosis score.

Changes in densitometric data during and after the combined therapy

Densitometric parameters reported at 24th week of therapy (29 patients) showed statistically significant improvement from the baseline. Further follow up DXA scan results at 48th week of therapy (25 patients) showed the same results as compared with the baseline parameters as well as those reported by the 24th week of therapy. This improvement was found to be sustainable by the 24th week after therapy, the reported densitometric data were still significantly better than the baseline parameters with no statistically significant difference from those reported at the 48th week of therapy (Table 3).

Seventeen patients (virological nonresponders) were withdrawn from the study at 12-week because discontinuation of treatment. Two patients of this group had "low BMD" and 5 patients had "at risk of low BMD"; at this point they were referred to Endocrinology Unit where they received appropriate managments. The remaining group of patients (n=10) showed normal densiometric parameters at baseline and they receive only routine care.

Discussion

It is well known that chronic liver disease affects the bone metabolism and BMD adversely.[14-16] Since the bone development during childhood and adolescence is the key determinant of adult skeleton health,[33] the current study aims at assessing BMD in children with non-cirrhotic CHC infection at diagnosis, during PEG-IFNα and RV therapy and at 6 months after end of treatment.

In our study, normal BMD was reported in 30 of 46 children with CHC at baseline; 10 patients were at risk for low BMD; and only 6 (13%) children had low BMD. Thess results were in agreement with those observed by Mora et al,[21] who found no significant difference in BMD between chronically infected untreated children with HCV and HBV infections and healthy controls.

The peak bone mass is normally achieved by late adolescence and early adulthood,[33] however, in our study children with low BMD were older than the rest of the study population. The reduction in the BMD in these patients may be, partialy, related to delayed puberty which was reported in most of them (5 out of 6 patients) that may be attributed to their past medical illnesses. Half of patients with low BMD experienced malignant tumors (two patients with acute lymphoblastic leukemia, one patient with lymphoma). Delayed puberty together with other endocrinal deficits are well recognized outcome in 40%-60% of pediatric patients who survived malignancy.[34] This pubertal delay may be secondary to malnutrition, emotional deprivation, side effects of chemotherapy[35] and possible hypothalamo-piuitary gonadal axis abnormalities related to high dose cranial irradiation.[34,36]

Furthermore, a strong relationship was described between malignancies and osteoporosis owing to cancer itself, decreased physical activity and chemotherapeutic agents.[37] In acute lymphoblastic leukemia, leukemic cells may infiltrate bone and secrete PTH and PTH related peptides causing bone resorption.[38,39] Also corticosteroids and methotrexate inhibit osteoblastic activity as well as increase osteoclastic bone resorption.[40,41] High doses of both agents tend to cause persistent decrease in BMD even after completion of chemotherapy.[42,43]

Many studies on adult populations showed reduction in BMD among patients with HCV infection.[44,45] However, this may be due to involvement of cirrhotic patients in most of these studies, while in non-cirrhotics, only few studies reported affection in BMD.[46-49] Moreover, a study on CHC well-nourished patients with preserved liver function, revealed no significant bone alterations.[50]

None of the liver related parameters such as histology activity index, fibrosis score or HCV RNA load was significantly correlated with BMD. This is consistent with Bunchorntavakul et al[20] who did not find any correlation between BMD and HCV RNA or predictors of the degree of necroinflammation, fibrosis and steatosis on liver histology. In contrast, Schiefke et al[46] had observed significant reduction in BMD in non-cirrhotic chronic hepatitis B or C infection that is proportionate to the advance in hepatic fibrosis. The reason of this controversy may be the presence of patients with advanced fibrosis in the later who were absent in the former studies like the situation in our series.

An interesting finding in the current study is that all densitometry parameters showed significant increment during and after PEG-IFN α and RV therapy compared

with the baseline results. The possible explanation is that IFNα has positive effect on bone metabolism; however the exact mechanism is not fully understood.[51] These results are supported by the finding of Bunchorntavakul et al[20] who found significant increase in BMD after treatment of CHC patients with combined IFN α and RV. Also, Hofmann et al[47] found a significant increase of BMD in CHC adult patients without established cirrhosis treated with PEG-IFNα and RV, which may last in patients with SVR.

On the contrary, Solís-Herruzo et al[19] had reported lower BMD in adult male patients receiving RV and IFN α than those receiving IFN α only and they suggested that RV was responsible for this adverse effect. However, it was a cross-sectional study which did not evaluate patients before treatment making these results inconclusive. Moreover, in a series of 20 pediatric patients with chronic HCV infection, Urganci et al[24] did not find any ribavirin-dependent changes related to bone mineral metabolism. Fortunately in our study chronic HCV infection led a relatively benign course as none of our patients had marked inflammation nor advanced fibrosis, also liver related laboratory results were satisfactory. Furthermore, we did not face major complication during treatment with PEG-IFNα and RV such as psychosis or depression that may affect nutrition or physical activity of the patients.

In conclusion, low BMD can be reported in a few percentages of pediatric CHC cases, particularly older children with delayed puberty and other comorbidities. Treatment with PEG-IFN α and RV had a sustainable positive impact on BMD, thus DXA scanning is not requested to monitor treatment side effects.

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Ethical approval: The principles outlined in the Declaration of Helsinki were followed and informed consents were obtained after study protocol approved by the local Ethics Committee of Mansoura University, Faculty of Medicine, Egypt.

Competing interest: All authors have no conflicts of interest.

Contributors: Megahed A suggested the idea, participated in study design, patients selection and treatment monitoring. Salem N participated in study design, organization and interpretation of DXA scanning, writing the primary draft. Fathy A shared in writing the primary draft, patients selection and follow-up. Barakat T shared in data collection and interpretation, patients selection and follow-up. Alsayed MAEL contributed to the study design, data collection and interpretation, patients selection and follow-up. Mabood SAE participated in writing the primary draft, patients selection and follow up. Zalata KR contributed to pathology reporting of obtained liver biopsies, data analysis. Abdalla AF supervised and organized the work, and participated in study design. All authors revised and approved the final version of the manuscript.

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