PEDIATRIC SCIENCES JOURNAL
The Official Journal of the Pediatric Department, Faculty of Medicine, Cairo University, Egypt

Original Article

Reduced Serum Glucagon Like Peptide-1 In Children with Osteoporosis of Chronic Liver Diseases: A Single Center Trial

Nashwa F. Mohamed Elmetwaly[1], Ola G. Behairy[1], Manal S. EL-Defrawy[1], Ola E. Seleim[2]*, Dina S. Abdelmotaleb[3]

[1] Department of Pediatrics, Faculty of Medicine, Benha University, Egypt
[2] Department of Pediatrics, Ain Shams General Hospital, Egypt
[3] Department of Clinical and Chemical Pathology, Faculty of Medicine, Benha University, Egypt
* Correspondence: olaessam990@gmail.com
Received: 30/8/2024; Accepted: 26/11/2024; Published online: 1/1/2025

Abstract:
Background: Chronic liver disease (CLD) in children is associated with reduction of bone mineral density. Glucagon like peptide- 1 (GLP-1) is essential for bone metabolism and bone turnover. GLP-1 role in bone disease associated with CLD remains to be studied.
Aim of the work: to study the relationship between GLP- 1 and osteoporosis among children with CLD.
Subjects and Methods: This cross-sectional study included 60 children with CLD as a study group and 60 healthy participants as a control group. GLP- 1 was measured using ELISA technique and compared between groups. All children with CLD underwent liver biopsy and bone density dual-energy X-ray absorptiometry (DEXA) scan. The study was conducted at Benha University Hospital, Egypt.
Results: The mean ± SD age of the included children with CLD and control group was 9.3 ± 5.1 years and 10.43 ± 5.54 years (p=0.130). Females and males comprised 33 (55%) and 27(45%) of the CLD group and 41 (68.3%) and 19 (31.7%) of the control group (p=0.133). The mean ± SD duration of liver diseases was 7.14 ± 4.51 years. Osteoporosis was encountered among 53 (88.3%) children with CLD. Their mean ± SD age was 9.51 ± 5.27 and their mean ± SD disease duration was 7.22 ± 4.65 compared to 7.71 ± 3.13 SD and 6.60 ± 3.52 SD of those who did not have osteoporosis (p=0.498) and (p=0.910) respectively. The mean± SD bone mineral density (BMD) and Z-score for lumber spine in children with CLD was 0.46 ± 0.14 g/cm^2 and mean± SD Z- score was -2.7 ± 0.38. BMD correlated negatively with liver disease duration: r: -0.135, p=0.303, histological activity index: r: -0.101, p=0.441, fibrosis: r: -0.046, p= 0.726, PELD: r= -0.46; p= 0.003; MELD: r= -0.71; p< 0.001; CHILD Pugh: r= -0.26; p= 0.04). The mean ±SD serum GLP-1 among children with CLD was 3.06 ± 1.07 pg/ml and 6.5± 2.01 pg/ml in the control group (p= 0.001). Serum GLP-1 correlated negatively with progressive fibrosis (p=0.008). Serum GLP-1 correlated with BMD (p=0.013), and at a cut-off value of 4 pg/mL, GLP- 1 had 86.7% sensitivity and 83.3% specificity in diagnosis of osteoporosis in children with CLD. Serum 25(OH) vit D less than 28 nmol/L had a 100% sensitivity and specificity for detection of osteoporosis.
Conclusion: DEXA confirmed osteoporosis of lumbar vertebrae among children with CLD. Serum GLP- 1 level was reduced among children with CLD. Serum GLP- 1 correlated inversely with degree of liver fibrosis and histological activity index and positively with the progression of osteoporosis in children with CLD. Low serum 25(OH) vit D is a sensitive and specific diagnostic marker of osteoporosis in CLD.

Keywords: children; GLP-1; chronic liver disease; liver fibrosis; bone density dual-energy X-ray absorptiometry; DEXA scan; bone mineral density; BMD
Abbreviations: BMD: bone mineral density; CLD: chronic liver disease; DEXA; dual-energy X-ray absorptiometry; GLP-1: glucagon like peptide -1 ; MELD: Model for end-stage liver disease; PELD: pediatric end-stage liver disease; MASH: metabolic associated steato-hepatitis

Introduction

Chronic liver diseases (CLD) encompass a spectrum characterized by the ongoing degeneration and regeneration of liver parenchyma, culminating in fibrosis, cirrhosis, and eventual hepatic failure. CLD is associated with complications, substantial morbidities, escalating public health challenge, and mortality (*1*). Osteoporosis, which results in high risk

fragility fractures, is a frequently observed complication in children with CLD, especially in liver cirrhosis (*2*, *3*)**.** Osteoporosis is a disease caused by an imbalance in the activities of osteoblasts and osteoclasts. When the balance between osteoclasts and osteoblasts is disrupted and bone resorption occurs more rapidly than bone formation, the amount of bone decreases and osteoporosis progresses**.** The International Society of Clinical Densitometry (ISCD) defined paediatric osteoporosis as ≥2 long bone fractures before the age of 10 years or ≥3 long bone fractures before the age of 19 years in combination with a low bone mineral density (BMD) for age and sex (z-score ≤−2.0 as measured by DXA), or ≥1 vertebral compression fractures (VF), independent of the BMD, in the absence of major trauma or local disease (*4*). Dual-energy X-ray absorptiometry (DEXA) is the preferred method for measuring BMD in children and adolescents and are presented as bone mineral content (BMC) (g/cm^2), depending on the bone mass Z-score generated by the number of SDs from the predicted mean, these results are compared to reference values from healthy kids of same age mass and sex (*4*). A lot of factors are incriminated as causes for the osteoporosis in CLD in children. Glucagon-like peptide 1 (GLP-1) promotes osteogenic differentiation, and its reduction was found to be associated with osteoporosis in adults. GLP-1, a peptide derived from the gastrointestinal tract, is synthesized via the post-translational modification of proglucagon, mediated by proprotein convertase subtilisin/kexin type 1 or 3 (PCSK1/PCSK3). This peptide manifests in two bioequivalent isoforms: the glycine-extended GLP-1 (GLP-1 7–37) and the amidated form, GLP-1 (GLP-1 7–36) (*5*). The physiological effects of GLP-1 are facilitated through its receptor, GLP-1R, which exhibits widespread expression across multiple tissues. GLP-1 is a strong inhibitor of glucagon secretion and enhances osteoblast proliferation, upregulates bone formation gene expression, and increases serum levels of bone formation markers, thereby promoting osteogenesis (*6*). Owing to local degradation of GLP-1 by the enzyme dipeptidylpeptidase-4 (DPP4) and further degradation in the liver, only 10–15% of endogenously released GLP1 reaches the systemic circulation (*7*). Limited studies have explored the relationship of GLP- 1 to osteoporosis among children with CLD. Thus, we aimed to study the relationship between GLP- 1 and osteoporosis among children with CLD.

Subjects and Methods

This cross-sectional case control study was conducted at the Paediatric Hepatology Unit of Benha University Hospitals in Benha, Egypt. The study was approved by the Ethical Committee of the Faculty of Medicine, Benha University, Egypt (MS-29-9-2021), affirming its adherence to The Code of Ethics of the World Medical Association (Declaration of Helsinki) (*8*). Prior to their consent, parents and guardians were provided with detailed information regarding the study's scope and purpose All the caregivers consented to the study. The study commenced March 1, 2022 and ended by March 6, 2023.

Participants

Sixty children with confirmed CLD diagnosis were included in the study and a control group that comprised 60 healthy, age- and sex-matched children attending routine check-ups for sports and school physicals. CLD was defined as a disease duration exceeding six months or by the presence of physical manifestations such as hepatosplenomegaly, spider telangiectasia, and clubbing. These indicators of chronicity were corroborated by laboratory, clinical and histopathological assessments as autoimmune hepatitis, chronic hepatitis C or B, cholestatic liver diseases, and metabolic hepatic disorders. Exclusion criteria encompassed CLD patients with concurrent conditions such as cardiovascular disease, renal disorders, inflammatory bowel disease, intestinal malabsorption, central nervous system dysfunction, etc.

Methods

All the participants were subjected to full history taking, clinical examination and laboratory assessment of complete blood count (CBC), liver function tests, serum calcium, 25 hydroxy vitamin D3 (25 -(OH) D) and serum GLP-1. The severity of liver disease was quantified using Child-Pugh score 8, model for end-stage liver disease (MELD) score 9 for teens older than 12 years of age, and pediatric-related end-stage liver disease (PELD) score 10 for participants younger than 12 years of age. Scores were calculated using the MDCalc medical calculator application (MD Aware, LLC, New York, NY). Mean ± SD PELD score was 7.2 ± 2.57, mean± SD MELD score was 14.5 ± 3.02 and mean± SD CHILD Pugh score was 7.55 ± 0.75 (*9*).

Lab Tests

Fasting venous blood samples were collected under stringent aseptic conditions to conduct CBC, coagulation profiles, including partial thromboplastin time (PTT), prothrombin time (PT), the international normalized ratio (INR), biochemical assays of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total protein, albumin, total and direct bilirubin; total and ionized calcium; alkaline phosphatase (ALP); 25-(OH) D; markers for hepatitis; autoantibodies; and human GLP-1.

- CBC was performed on all specimens using the XS series automated haematology analyzer, model SN 12526, manufactured by SYSMEX Corporation in Kobe, Japan. Subsequently, blood smears were prepared and stained using Leishman's stain to facilitate a differential count.
- Coagulation functions (PT, PTT and INR): using Automated Blood Coagulation Analyzer CS-1600, SN 12058, Sysmex Corporation, Kobe, Japan.
- Biochemical liver function tests: AST, ALT, ALP, total protein, albumin and bilirubin (total and direct) were assessed by DIALAB, 13771103, Thermo company, USA.
- GGT was assessed by INDIko, SN 864000003200, Thermo company, USA.
- Serum calcium (total and ionized) and 25-(OH) D were assessed by ST 200 Plus, Sensacore, SN IVD5214732, Thermo company, USA.
- Serum concentrations of Human Glucagon-like peptide-1 were quantified using ELISA kits supplied by Sunred Biotechnology Company (Catalog No. 201-12-0023), following the procedural guidelines provided by the manufacturer. The measurements were conducted on a DAS plate reader, model SN 1912, located in Palombara Sabina, Italy.

Liver Percutaneous Biopsy

Within the cohort of CLD patients, liver biopsies were collected under ultrasound guidance, employing Menghini aspiration needles (Hepafix Luer Lock Braun Melsungen AG, Melsungen, Germany). Liver specimens were extracted to guarantee the inclusion of a minimum of 11 portal tracts, and they were subsequently preserved in formalin. The Ishak scoring system and histological activity evaluation were employed to evaluate liver fibrosis staging by staining tissue sections with eosin and hematoxylin. Fibrosis was categorized according to the following criteria: F0 denoted the absence of fibrotic changes; F(1-2/6) indicated fibrous expansion within some or most portal areas, possibly accompanied by short fibrous septa; F(3-4/6) reflected extensive fibrous enlargement across most portal regions, with either occasional or substantial bridging (both portal-portal and portal-central); and F(5-6/6) described pronounced bridging (portal-portal and/or portal-central), accompanied by intermittent nodular formations (indicative of incomplete cirrhosis) or the presence of fully established cirrhosis (*10*). Additional stains such as Mason-Trichrome for fibrosis depth, Perls' Prussian blue for iron accumulation, and periodic acid-Schiff for alpha-1 antitrypsin deficiency were also employed to provide a comprehensive analysis of hepatic pathology.

Imaging Studies

Children with CLD underwent abdominal ultrasound using Mindray ultrasound machine, Mindray global company, China and Dual Energy X-ray Absorptiometry (DEXA) scan lumber spine for all children with CLD using Medix 90 DEXA Bone Densitometer, manufactured by Medilink company, France. BMD was assessed in children with CLD by DEXA scan that measures bone mineral content in grams and bone area in square centimeter then calculates “a real” BMD in g/cm^2 by dividing bone mineral content and bone area. BMD is a DEXA derived measure calculated by dividing Bone Mineral Content (BMC) (in grams) by projected bone surface area (in cm^2) (*11*). The Z- score to compare BMD in the children with CLD to a population of peers, was calculated by subtracting the mean BMD of an age, ethnicity and sex matched reference population from the patient BMD and dividing by the SD of the reference population (*12*). The diagnosis of osteoporosis in children was made if there was a size-corrected low BMD (Z- score≤ -2 S.D) according to the International Society for Clinical Densitometry (ISCD) (*11*).

Statistical Analysis

The results were gathered and analysed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp, USA). Qualitative data were described using number and percent. The Kolmogorov test was used to assess data normality. Normally distributed continuous data were expressed as mean ± standard deviation. Appropriate statistical tests were used according to data type. Chi square test was used to compare categorical data. Student t- test was used to compare normally distributed data between 2 groups. Receiver operator characteristics test was used to assess the diagnostic performance of certain variables of continuous type. ROC curve analysis was employed to ascertain the serum GLP-1 threshold values conducive for the

diagnosis of both advanced and early fibrosis, ensuring optimal specificity and sensitivity. Multivariate regression analysis was utilized to forecast fibrosis scores. Statistical significance was established at a p-value of 0.05 within a 95% CI.

Results

Table 1. Laboratory and DEXA findings among the studied groups

		Liver Disease Group (n = 60)	Control group (n = 60)	Test of Sig.	P
Total bilirubin (mg/dl)	Range	2.0 – 3.70	0.20 – 0.90	U=0.0*	<0.001
	Mean ± SD.	3.01 ± 0.43	0.63 ± 0.19		
	Median (IQR)	3.0 (2.70 – 3.40)	0.70 (0.50 – 0.80)		
Direct bilirubin (mg/dl)	Range	0.50 – 1.70	0.02 – 0.30	U= 0.0*	<0.001
	Mean ± SD.	0.69 ± 0.23	0.13 ± 0.07		
	Median (IQR)	0.60 (0.55 – 0.70)	0.12 (0.08 – 0.20)		
AST (U/l)	Range	35.0 – 640.0	11.0 – 40.0	U= 8.50	<0.001
	Mean ± SD.	152.3 ± 119.8	24.82 ± 8.35		
	Median (IQR)	94.50 (71.0 – 191.5)	22.0(20.0 – 33.0)		
ALT (U/l)	Range	72.0 – 650.0	15.0 – 50.0	U= 0.0	<0.001
	Mean ± SD.	138.9 ± 106.3	30.25 ± 11.34		
	Median (IQR)	95.0 (86.50 – 143.0)	31.50 (18.0 – 38.0)		
ALP (U/l)	Range	100.0 – 1233.0	1.0 – 91.0	U= 0.000	<0.001
	Mean ± SD.	317.2 ± 279.4	36.27 ± 24.26		
	Median (IQR)	200.0 (130.0 – 365.0)	33.0 (18.0 – 55.50)		
GGT (U/l)	Range	23.0 – 401.0	8.0 –25.0	U= 95.50	<0.001
	Mean ± SD.	72.77 ± 89.09	18.27 ± 10.41		
	Median (IQR)	40.0 (35.0 – 49.50)	16.50(13.0 – 20.0)		
Serum total protein (g/dl)	Range	4.20 – 6.0	3.50 – 5.50	t= 7.660	<0.001
	Mean ± SD.	5.39 ± 0.58	4.54 ± 0.64		
	Median (IQR)	5.50 (4.80 – 6.0)	4.80 (3.80 – 5.0)		
Serum Albumin (g/dl)	Range	2.20 – 3.50	2.30 – 4.80	U= 189.0	<0.001
	Mean ± SD.	2.81 ± 0.29	3.95 ± 0.55		
	Median (IQR)	2.90 (2.50 – 3.0)	4.0 (3.50 – 4.30)		
PT (seconds)	Range	11.0 – 21.0	11.0 – 14.0	T= 2.088	0.04
	Mean ± SD.	13.16 ± 1.84	12.60 ± 0.96		
	Median (IQR)	13.0 (12.0 – 14.0)	12.60 (11.8 – 13.0)		
PTT (seconds)	Range	14.0 – 55.0	14.0 – 46.0	t= 2.365	0.02
	Mean ± SD.	36.10 ± 7.0	32.95 ± 7.58		
	Median (IQR)	37.0 (33.0 – 40.0)	32.50 (27.0 – 38.0)		
INR	Range	1.0 – 2.0	0.50 – 1.0	U= 63.0	<0.001
	Mean ± SD.	1.19 ± 0.32	0.73 ± 0.15		
	Median (IQR)	1.10 (1.0 – 1.20)	0.70 (0.60 – 0.80)		
HB (g/dl)	Range	8.0 – 12.10	8.30 – 14.0	t= 2.839	0.005
	Mean ± SD.	10.25 ± 1.27	11.01 ± 1.65		
	Median (IQR)	10.0 (9.0 – 11.25)	11.45 (9.0 – 12.0)		
WBCs ($\times 10^3/\mu l$)	Range	3.30 – 20.0	4.70 – 17.0	U= 1564.00	0.213
	Mean ± SD.	8.60 ± 3.96	9.3 ± 3.28		
	Median (IQR)	8.0 (5.40 – 10.90)	10.0 (6.25 – 10.90)		
PLTs ($\times 10^3/\mu l$)	Range	140.0 – 400.0	27.0 – 620.0	U= 1786.50	0.943
	Mean ± SD.	285.4 ± 82.35	307.2 ± 151.6		
	Median (IQR)	300.0 (204.0– 360.0)	300.0 (199.5– 480)		
Total serum calcium	Range	6.20 – 9.0	8.0 – 9.90	13.936	<0.001
	Mean ± SD.	7.68 ± 0.53	9.05 ± 0.54		
	Median (IQR)	7.90 (7.50 – 8.0)	9.05 (8.60 – 9.50)		
Ionized serum calcium	Range	0.80 – 2.40	1.80 – 3.40	12.810	<0.001
	Mean ± SD.	1.50 ± 0.45	2.62 ± 0.51		
	Median (IQR)	1.50 (1.20 – 1.90)	2.50 (2.20 – 3.0)		
25(OH)Vit D(nmol/L)	Range	15.0 – 28.0	50.0 – 69.0	52.864	<0.001
	Mean ± SD.	20.13 ± 3.10	57.27 ± 4.47		
	Median (IQR)	19.25 (18.0 – 22.0)	57.75 (53.5 – 59.0)		
Serum GLP-1 (pg/mL)	Range	1.80 – 6.0	3.40 – 10.0	206.50	<0.001
	Mean ± SD.	3.06 ± 1.07	6.51 ± 2.01		
	Median (IQR)	2.70 (2.45 – 3.25)	6.95 (4.80 – 8.30)		
DEXA BMD	Range	0.31 – 0.87			
	Mean ± SD.	0.46 ± 0.14			
	Median (IQR)	0.41 (0.38 – 0.54)			
DEXA Z score	Range	-3.60 – -2.10			
	Mean ± SD.	-2.71 ± 0.38			
	Median (IQR)	-2.60 (-3.0 – -2.50)			

ALT: Alanine aminotransferase; ALP=Alkaline phosphatase; AST: Aspartate Aminotransferase; BMD=Bone Mineral Density; DEXA: Dual-Energy X-Ray Absorptiometry; 25(OH)Vit D: 25-hydroxy vitamin D; GGT: Gamma glutamyl transferase; INR: International Normalized Ratio; PT: Prothrombin Time, PTT: Partial Thromboplastin Time; HB: Hemoglobin; WBCs: White Blood Cells; PLTs: Platelets; GLP-1: glucagon like peptide-1.

This study included 120 children. Of these, 60 children with confirmed CLD diagnosis. This group included 27 (45%) males and 33 (55%) females, with a mean age of 9.30 ± 5.08 years (range= 1.0 to 18.0 years). The control group consisted of 60 healthy sex and age matched children, of them 19 boys (31.7%) and 41 (68%) girls with a mean age of 10.43 ± 5.54 years (range 1 to 18.0 years) (p=0.130) and (p=0.133) respectively. History of consanguinity was comparable among both groups (p=0.714). However, a statistical disparity was noted in the prevalence of positive family history of CLD within the study group compared to the controls (p = 0.001). Positive family history of CLD was present in 13 (21.7%) of the children with CLD, while none of the control group had positive family history of CLD.

Clinical manifestations observed in cases of CLD predominantly included abdominal distension, reported in 35 (58.3%) of patients, followed by jaundice in -17(28.3%), abdominal pain in 7(11.7%), and lower limb edema in 1 (1.7%). Clinically 38 (63%) of the diagnosed individuals exhibited hepatomegaly, while 31 (51.6%) presented with splenomegaly. The etiologies of CLD within the studied cohort were diverse, with glycogen storage disease in 17 (28.4%), chronic hepatitis C virus infection in 13 (21.7%), chronic hepatitis B virus in 11 (18.3%), metabolic associated steato-hepatitis (MASH) in 6 (10%), biliary atresia in 5 (8.3%), autoimmune hepatitis in 5 (8.3%), and congenital hepatic fibrosis in 2 (3.3%) and Dubin-Johnson syndrome in 1 (1.7%). The mean age at onset of liver disease symptoms was 2.13 ± 2.31 years, and the average duration of illness was 7.14 ± 4.51 years. The initial laboratory results for the study participants are detailed in Table 1. Notably, the serum GLP-1 concentration was found to be significantly reduced in individuals with CLD compared to the healthy control group (p= 0.001). The histopathological evaluation of liver biopsies, as classified by the Ishak scoring system, demonstrated that disease activity was minimal in 6 (10%) of the subjects, mild in 36 (60%), moderate in 13 (21.7%), and severe in 5 (8.3%) of the participants. The extent of fibrosis varied, with mild fibrosis (F1-2/6) observed in 29 (48.3%) of patients, moderate fibrosis (F3-4/6) in 20 (33.3%), and severe fibrosis (F5-6/6) in 11 (18.3%) cases. In terms of cellular infiltration, eosinophils were detected in 9 (15%) of the cases, lymphocytes in another 9 (15%), plasma cells constituted 7 (11.7%), and mononuclear inflammatory cells were the most prevalent, occurring in 35 (58.3%) of the biopsies (Table 2).

Table 2. Liver histology among the children with chronic liver disease

			N	%	25(OH)Vit D (nmol/L)	Serum GLP-1 (pg/ml)	DEXA BMD	DEXA Z-score
					Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Degree of fibrosis	Mild	1-2/6	29	48.3	20.47 ± 3.38	3.21 ± 1.07	0.47 ± 0.16	-2.70 ± 0.39
	Moderate	3-4/6	20	33.3	19.98 ± 2.24	3.14 ± 1.09	0.46 ± 0.14	-2.77 ± 0.44
	Marked	5-6/6	11	18.3	19.55 ± 3.80	2.49 ± 0.89	0.42 ± 0.08	-2.63 ± 0.23
	P value				0.685	0.008	0.860	0.554
Histological activity index	Minimal	1-3/8	6	10.0	18.58 ± 2.33	3.63 ± 1.59	0.47 ± 0.19	-2.87 ± 0.45
	Mild	4-8/18	36	60.0	20.74 ± 3.08	3.26 ± 1.06	0.48 ± 0.16	-2.72 ± 0.39
	Moderate	9-12/18	13	21.7	19.85 ± 3.47	2.57 ± 0.58	0.42 ± 0.07	-2.69 ± 0.39
	Marked	13-18/18	5	8.3	18.40 ± 2.30	2.18 ± 0.43	0.40 ± 0.09	-2.46 ± 0.17
	P value				0.211	0.007	0.658	0.317
Type of infiltrating cells	Eosinophils		9	15.0	20.0 ± 2.35	2.61 ± 0.55	0.35 ± 0.04	-2.64 ± 0.18
	Lymphocytes		9	15.0	19.56 ± 3.10	3.30 ± 1.10	0.41 ± 0.09	-2.81 ± 0.44
	Plasma		7	11.7	20.93 ± 3.61	3.39 ± 1.58	0.47 ± 0.17	-2.86 ± 0.43
	Mononuclear		35	58.3	20.16 ± 3.26	3.04 ± 1.04	0.49 ± 0.15	-2.67 ± 0.39
	P value				0.858	0.850	0.008	0.523

BMD: Bone Mineral Density; DEXA: Dual-Energy X-Ray Absorptiometry; 25(OH)Vit D: 25-hydroxy vitamin D; GLP-1: glucagon like peptide-1.

Serum GLP-1 level was reduced in all cases with CLD, the least level was detected in those with auto immune hepatitis (mean ± SD= 2.62 ± 0.64) and highest level was detected in those with congenital hepatic fibrosis (mean ± SD= 4.30 ± 2.40) (p= 0.945). (Table 3).

Osteoporosis was encountered among 53 (88.3%) children with CLD. Their mean ± SD age was 9.51 ± 5.27 and their mean ± SD disease duration was 7.22 ± 4.65 compared to 7.71 ± 3.13 SD and 6.60 ± 3.52 SD those who did not have osteoporosis (p=0.498) and (p=0.910) respectively. The mean ±SD DEXA Scan Z-score for lumber spine was less than -2 in all cases of our study, with different chronic liver diagnoses as follows: in auto immune hepatitis= -2.64 ± 0.17, glycogen storage disease= -2.68 ± 0.42, chronic viral hepatitis= -2.69 ± 0.39, congenital hepatic fibrosis= -

2.75 ± 0.35, MASH= -2.77 ± 0.51 and biliary atresia= -2.78 ± 0.33 (p=0.842). (Tables 3 and 5). Bone density correlated negatively with the severity of liver diseases as measured by PELD score (p=0.003) and the MELD score (p=0.001) and did not corelate with the Child-Pugh score (p=0.047). Serum GLP- 1 correlated negatively with liver span (p=0.025), spleen dimensions (p=0.011), AST (p=0.037), ALT (p=0.001), PELD (p=0.016), MELD (p=0.001), CHILD Pugh (p=0.018) degree of fibrosis (p=0.008) and histological activity index (p= 0.007) (Tables 4 and 5).

Table 3. Serum GLP-1 level (pg/mL) and DEXA Scan Z- score in those with chronic liver diseases

	N	Serum GLP-1 (pg/mL)		P value	DEXA Scan Z-score		P value	Bone mineral density of Lumbar spine		P value
		Mean ±SD	Range		Mean ± SD	Range		Mean ± SD	Range	
MASH	6	3.38 ±1.79	1.9 – 6.0		-2.77 ±0.51	-3.5 – -2.20		0.49 ± 0.17	0.31 – 0.70	
Auto immune hepatitis	5	2.62 ±0.64	2.0 – 3.5		-2.64 ±0.17	-2.9 – -2.50		0.40 ± 0.09	0.31 – 0.56	
Chronic viral hepatitis	24	3.18 ±1.08	1.9 – 6.0		-2.69 ±0.39	-3.5 – -2.10		0.44 ± 0.13	0.31 – 0.87	
Glycogen storage disease	17	2.76 ±0.57	1.8 – 4.0	0.842	-2.68 ±0.42	-3.6 – -2.10	0.945	0.47 ± 0.16	0.31 – 0.87	0.294
Biliary atresia	5	3.05 ±1.09	2.5 – 5.0		-2.78 ±0.33	-3.3 – 2.5		0.51 ± 0.14	0.35 – 0.70	
Dubin Johnson Syndrome	1	2.90 *			-3.10 *			0.31		
Congenital hepatic fibrosis	2	4.3 ± 2.40	2.6 – 6.0		-2.75 ± 0.35	-3.0 – -2.5		0.45 ± 0.14	0.35 – 0.54	

DEXA: Dual-Energy X-Ray Absorptiometry; GLP-1: glucagon like peptide-1; MASH: Metabolic Associated Steato-Hepatitis; p value: probability value; SD: standard deviation; *: Excluded from the comparison being a single number of case (n = 1)

Table 4. Correlation of serum GLP-1, bone mineral density score and Z- score to PELD, MELD and Child- Pugh scores according to clinical and lab findings of chronic liver disease

	Serum GLP-1 (pg/ml)		**DEXA BMD**		**DEXA Z-score**	
	r	**P**	**r**	**P**	**r**	**P**
Age at study (years)	0.107	0.418	-0.156	0.233	0.128	0.331
Age of onset of liver disease (years)	-0.159	0.224	-0.052	0.693	0.053	0.688
Duration of liver disease (years)	0.165	0.208	-0.135	0.303	0.129	0.325
Weight centile	0.076	0.566	-0.256	0.048*	-0.149	0.255
Height centile	0.001	0.992	0.109	0.408	0.222	0.089
BMI centile	0.073	0.580	-0.225	0.084	0.012	0.928
BMI Z-score	0.014	0.914	0.134	0.306	-0.010	0.940
Liver span (cm)	-0.289	0.025*	-0.182	0.164	0.215	0.099
Spleen size (cm)	-0.325	0.011*	-0.186	0.155	0.226	0.082
Total Bilirubin (mg/dl)	-0.149	0.257	-0.173	0.186	0.059	0.657
Direct Bilirubin (mg/dl)	0.217	0.095	-0.168	0.200	-0.136	0.301
AST (u/l)	-0.270	0.037*	0.129	0.325	0.121	0.358
ALT (u/l)	-0.423	0.001*	-0.068	0.608	0.229	0.078
ALP (u/l)	-0.101	0.443	0.120	0.359	0.104	0.430
GGT (u/l)	-0.108	0.412	-0.012	0.926	0.194	0.138
PT (sec)	-0.195	0.136	-0.267	0.039*	-0.063	0.635
PTT (sec)	0.024	0.855	-0.026	0.843	-0.010	0.940
INR	0.042	0.749	-0.152	0.245	-0.045	0.733
Serum total protein (g/dl)	0.002	0.991	0.001	0.994	0.075	0.569
Serum Albumin (g/dl)	0.328	0.010*	0.078	0.554	-0.129	0.325
HGB (g/dl)	-0.043	0.743	0.052	0.695	-0.078	0.551
WBCs ($\times 10^3/\mu l$)	0.091	0.490	0.068	0.606	-0.194	0.137
PLTs ($\times 10^3/\mu l$)	0.108	0.412	0.247	0.057	0.036	0.786
Total calcium (mg/dl)	0.267	0.039*	0.033	0.803	-0.141	0.284
Ionized calcium (mg/dl)	-0.315	0.014*	-0.319	0.013*	0.225	0.084
25(OH)Vit D (nmol/l)	0.309	0.016*	0.032	0.807	-0.232	0.075
Degree of fibrosis	0.046	0.724	0.076	0.563	-0.029	0.824
Histological activity index	-0.416	0.001	-0.101	0.441	0.188	0.149

ALT: Alanine aminotransferase; ALP=Alkaline phosphatase; AST: Aspartate Aminotransferase; BMD: Bone Mineral Density; BMI: Body Mass Index; DEXA: Dual-Energy X-Ray Absorptiometry; 25(OH)Vit D: 25-hydroxy vitamin D; GGT: Gamma Glutamyl Transferase; GLP-1: glucagon like peptide-1; HB: Hemoglobin; INR: International Normalized Ratio; P value**:** probability value; PLTs: platelets; PT: Prothrombin Time; PTT: Partial Thromboplastin Time; r: Spearman coefficient; WBCs: White Blood Cells. Spleen size was measured in centimeters below costal margin.

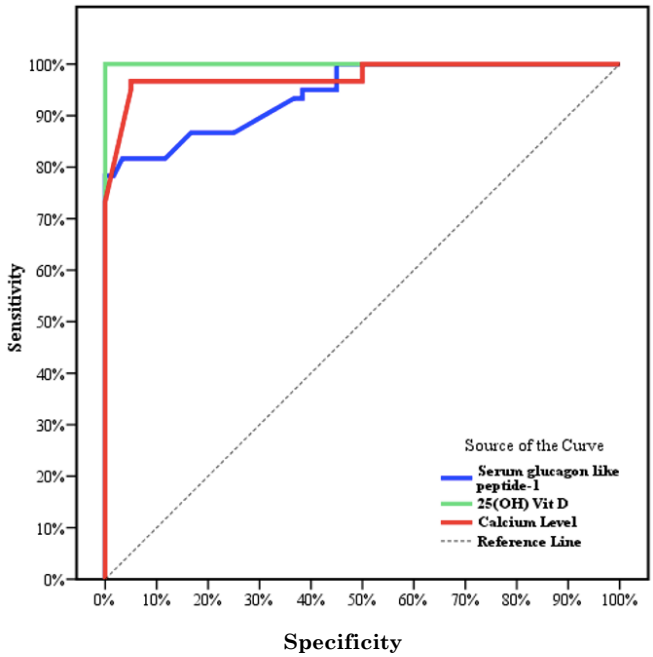
Serum GLP- 1 correlated positively with serum albumin (p=0.010), total and ionized calcium (p=0.039 and 0.014) respectively, and 25-(OH) D (p=0.016) concentrations, with BMD (p=0.013) and z-scores (p=0.014) (Table 4). Serum GLP- 1 did not correlate with hemoglobin levels (p=0.743), GGT (p=0.412) or BMI centile (p=0.580). The cut-off of GLP-1 level in diagnosis of osteoporosis among children with CLD was 4 pg/mL (sensitivity of 86.67% and specificity of 83.33% (area under the ROC curve (AUC) =0.943). (Table 6 and Figure 1).

Table 5. Correlation of bone mineral density score, Z- score and serum GLP-1 to PELD, MELD and Child- Pugh scores according to etiology of chronic liver disease

	Bone mineral density [Lumbar spine] (g/cm²)		DEXA Z-score		Serum GLP-1 (Pg/mL)	
	Mean	SD	MEAN	SD	Mean	SD
	r	P	r	P	r	P
PELD score	-0.464	0.003	-0.515	0.001	-0.378	0.016
MELD score	-0.714	<0.001	-0.537	0.015	-0.695	0.001
Child Pugh score	-0.257	0.047	-0.179	0.171	-0.304	0.018

GLP-1: glucagon like peptide-1; MASH: Metabolic Associated Steato-Hepatitis; MELD: Model for End-Stage Liver Disease; PELD: Pediatric End-Stage Liver Disease; P value: probability value; r_s: Spearman coefficient.

Figure 1. Serum GLP-1 level and Vitamin D level in Prediction of DEXA Scan Z-score in the study group



	Cut off	Sensitivity	Specificity	PPV	NPV	P	AUC	95% C.I
Serum GLP-1 (pg/mL)	4	86.67	83.33	83.9	86.2	<0.001*	0.943	0.90 –0.98
25(OH) Vit D (nmol/L)	≤28	100.0	100.0	100.0	100.0	<0.001*	1.000	1.0 – 1.0
Calcium Level (mg/dl)	≤8.3	96.67	95.0	95.1	96.6	<0.001*	0.977	0.952 – 1.0

AUC: Area under the curve; CI: Confidence Interval; GLP-1: Glucagon like peptide -1; NPV: Negative Predictive Value; P value: probability value; PPV: Positive Predictive Value; 25(OH)Vit D: 25-hydroxy vitamin D

Table 6. Serum GLP-1 level and DEXA Scan Z-score according to treatment in the study group

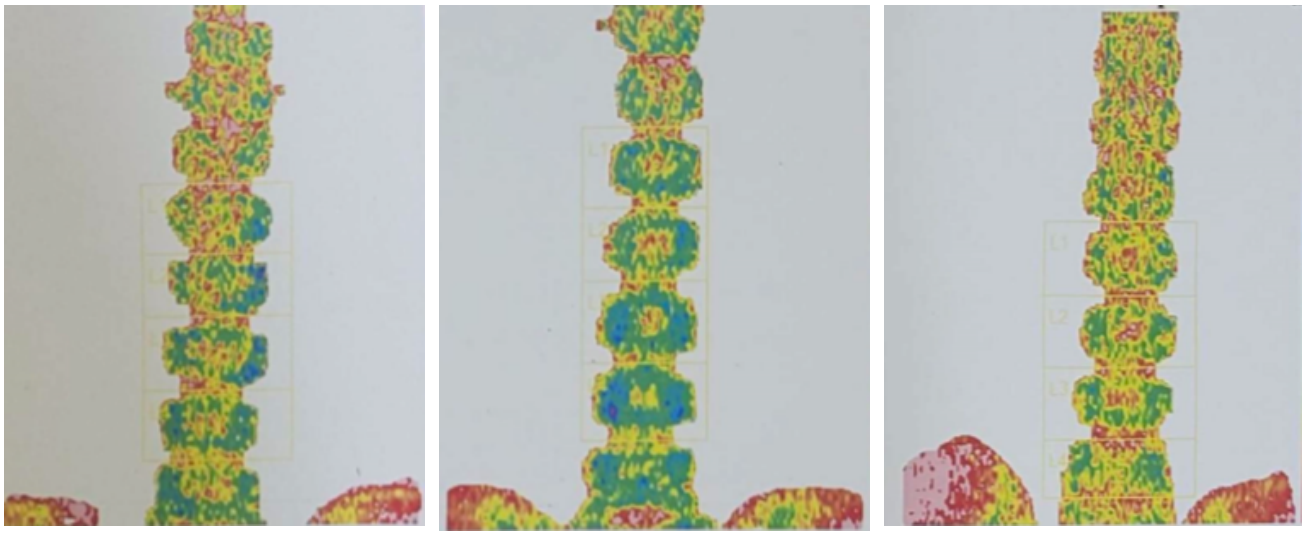
			Serum GLP-1		U-test	P value	DEXA Scan Z score		U-test	P value
	N	%	Mean ±SD	Range			Mean ± SD	Range		
No Medicine	6	10	3.03 ± 0.98	2.5 –5.0	162.0	1.000	-2.77 ± 0.51	-3.5 – -2.2	143.5	0.656
spironolactone	1	1.7	2.6#		-	-	-2.5		-	-
Antioxidants	6	10	3.38 ± 1.79	1.9 –6.0	143.0	0.656	-2.65 ± 0.34	-3.4 – -2.2	254.0	0.523
Direct-acting antivirals	12	20	3.11 ± 1.18	2.1 –6.0	285.5	0.963	-2.64 ± 0.17	- -2.9 – 2.5	136.0	0.979
Prednisone and azathioprine	5	8.3	2.62 ± 0.64	2.0 –3.5	113.0	0.532	-2.83 ± 0.32	-3.3 – -2.50	115.0	0.259
Starch	17	28.3	2.76 ± 0.57	1.8 –4.0	330.5	0.565	-2.68 ± 0.42	-3.60 – -2.1	324.5	0.494
Supportive therapy	1	1.7	6.0#		–	–	-2.75		–	–
Tenofovir	12	20	3.25 ± 1.02	1.9 –5.0	233.0	0.309	-2.73 ± 0.45	-3.5 – -2.10	281.0	0.895

DEXA: Dual-Energy X-Ray Absorptiometry; GLP-1: Glucagon Like Peptide-1; p value: probability value; U: Mann Whitney test; SD: standard deviation; #: Excluded being a single case (n = 1).

At cut-off value of 2.60, GLP- 1 had the ability to differentiate between mild and moderate to severe liver fibrosis with 72.4% sensitivity and 51.6% specificity and moderate AUC of ROC curve= 0.654. (Figure 1). The cut-off of 25-(OH) D level in diagnosis of osteoporosis among children with CLD was ≤ 28 nmol/L (sensitivity of 100% and a specificity of 100% (AUC ROC

curve = 1.000). (Table 6 and Figure 1). There was no correlation between 25-(OH) D level and DEXA scan findings BMD and Z- score (p= 0.328) and (p=0.328) respectively. (Table 6).

The group with CLD were on the following medications according to the definitive diagnosis and current illness: 17 cases (28.3%) were on starch for those with glycogen storage disease, 12 (20%) on tenofovir for hepatitis B virus, 12 (20%) on direct-acting antivirals (DAAs) for hepatitis C virus, 6 (10%) were on antioxidants with life style modification for those with MASH, 5 (8.3%) were on prednisone and azathioprine for those with auto immune hepatitis, 1 (1.7%) was on spironolactone, 1 (1.7%) was on supportive therapy (symptomatic treatment in form of endoscopy for treating varices, multivitamins for liver support, beta blocker for portal hypertension) and 6 (10%) of cases did not receive medications (Table 7). The comparison between DEXA scan Z. score of the cases and medications received by them is outlined in Table 7. Comparing the DEXA scan findings of the lumber (L) vertebrae in the group with CLD showed that the mean± SD DEXA Z. score for L1 = -1.96± 0.73, for L2 = -1.55 ± 0.68, for L3= -1.45± 0.72 and for L4 = -1.40 ± 0.91. The most affected lumber vertebra was L1. (Table 7). (Figure 2).



a **b** **c**

Figure 2. DEXA scan of a cases with chronic liver disease.

a) DEXA scan of lumbar vertebrae 1-4 of an 8 year-old boy with metabolic associated steato-hepatitis of 5 years duration. The BMD was 0.376 g/cm^2 and Z-score of -2.6, both were significantly lower than that expected for age and sex. b) DEXA scan of lumbar vertebrae 1-4 of an 11 year-old boy with glycogen storage disease of 10 years duration. The BMD was 0.547 g/cm^2 and Z-score of -1.2, both were lower than that expected for age and sex. c) DEXA scan of lumbar vertebrae 1-4 of an 8 year-old boy with biliary atresia of 7.9 years duration. The BMD was 0.382 g/cm^2 and Z-score of -2.5, both were significantly lower than that expected for age and sex.

Table 7. Comparison between DEXA scan Z-score of Lumbar vertebra 1, L2, L3 and L4 vertebrae of children with chronic liver disease

Z- score	L1	L2	L3	L4	Fr	P value
Range	-3.60 – -0.90	-2.60 – -0.20	-2.70 – -0.40	-4.20 – -0.30	98.96	<0.001
Mean ± SD	-1.96± 0.73	-1.55 ± 0.68	-1.45± 0.72	-1.40 ± 0.91		
Median (IQR)	-1.75 (-2.60 – -1.50)	-1.40 (-2.30 – -1.10)	-1.35 (-1.85 – -1.00)	-1.25 (-2.15 – -0.85)		
Significance	p_1<0.001*, p_2<0.001, p_3<0.001*, p_4=0.026*, p_5<0.001*, p_6=0.048*					

Fr: Friedman test, Significance between periods were done using Post Hoc Test (Dunn's)
p: p value for comparing between different L1, L2, L3 and L4; p_1: p value for comparing between different L1 and L2; p_2: p value for comparing between different L1 and L3; p_3: p value for comparing between different L1 and L4; p_4: p value for comparing between different L2 and L3; p_5: p value for comparing between different L2 and L4; p_6: p value for comparing between different L3 and L4

Discussion

Osteoporosis confirmed by DEXA scan complicated 53 (88.3%) of our studied children with CLD. Osteoporosis is a frequently observed complication in almost 40%- 70% of patients with chronic liver disease (*13*). The diagnostic accuracy increases with use of DEXA. There are common etiological factors related to chronic liver disease in general that affect bone metabolism.

These include vitamin D and calcium metabolism alterations, vitamin K deficiency, and hormonal dysregulation, the release of cytokines and deficiency of insulin-like growth factor 1 (IGF-1) (*14*). There was a negative correlation between DEXA scan confirmed bone mineral density and severity of liver diseases as PELD, MELD, while there was no statistical correlation with Child Pugh score.

Among our studied population osteoporosis was related to the severity of liver diseases as measured by PELD score (p=0.003) and the MELD score (p=0.001), weight centile (p= 0.048), ionized calcium level (0.013) and not related to age, duration of liver disease, height centile, liver span or spleen size. It is alarming however that the osteoporotic changes were irrespective of presence of cholestasis (p=0.657) which is said to impede fat soluble vitamin absorption. Our work supports the multifactorial aetiology of bone disease among those with CLD. Hence, vitamin D should be part of the treatment for any child with CLD. We did not study the exercise effect or school attendance or type or sedentary life dictated by the liver disease. It is to be noted that only one child was on diuretics, hence the role of diuretics seems not to be central as a cause of osteoporosis in CLD among children.

The main finding of this study was the alarming low GLP-1, calcium levels and deficient/insufficient 25(OH) vit D levels among our studied cohort with CLD. There was an association between low serum GLP-1 levels and osteoporosis in our studied children with CLD. It is not clear it this low level is associated with the liver disease or due to low vitamin D levels among our studied cohort. This is especially interesting as GLP-1 is degraded naturally by the liver (*7*), hence it would be expected to be higher among those with compromised liver functions. Hence, we suspect that this reduced GLP-1 might be related to the reduced vitamin D levels.

In our study, we found a statistically significant decrease in serum GLP-1 level in those with CLD than control group. The accepted golden standard for osteoporosis is DEXA scan, which bears irradiation exposure as the regular chest X-ray (*15*). Yet it has multiple short comings, the irradiation exposure, the age limit, and cost. Hence the need for other less invasive alternatives for diagnosis of osteoporosis is necessary. Serum GLP-1 level seems to have modest 86.67% sensitivity and 83.33% specificity to define those with CLD and osteoporosis, which renders serum GLP-1 a plausible factor within a scoring system. The accepted serum GLP-1 among healthy children is 0-15pg/mL, has a very short half-life of 2 minutes, and it increases after meals (*16*). Namely, if serum GLP-1 is not detected it might be a normal finding. Hence, the serum GLP-1 measurement standardization is imperative, and its value in diagnosis of osteoporosis remains to be studied**.** There is a conflicting role of GLP-1 agonist medications to promote bone health in osteoporosis (*17*), and its role children with CLD remains to be studied (*6*).

It is interesting, however, that among those with CLD, a level of 25(OH) vit D less than 28 nmol/L had a 100% sensitivity and specificity for detection of osteoporosis, which promises to preclude the need for DEXA imaging among children with CLD irrespective of the level of total bilirubin. It is interesting that our control group had a serum vitamin D level range of 50-69 nmol/L, which is higher than 94% of healthy Egyptian children and adolescents (*18*). More studies are needed to verify the daily recommended dose of vitamin D among those with CLD.

Our work provides evidence that supports the superior sensitivity and specificity of 25(OH) vit D to the serum GLP-1 assessment. The assessment of 25(OH) vit D is currently readily available commercially and easily interpreted. Hence, analysis of level of 25(OH) vit D and its supplementation should be part of the work up for any child with CLD. While it is known that vitamin D stimulates the production of GLP-1 among the elderly with fractures (*19*), it remains however to be seen if the supplementation of 25(OH) vit D would correct the bone disease and stimulate production of GLP-1, and the subsequent enhancement of bone remodeling among those with CLD.

It is alarming, however, that our studied cohort had significantly low calcium levels, it remains to be studied if vitamin D and calcium supplementation is necessary among children with CLD, or vitamin D supplementation alone would correct the calcium level, given that calcium is not always enough in diet (*20*).

Conclusion

DEXA confirmed osteoporosis of lumbar vertebrae among children with CLD. Serum GLP- 1 level was reduced among children with CLD. Serum GLP- 1 correlated inversely with degree of liver fibrosis and histological activity index and positively with the progression of osteoporosis in children with CLD. Low serum 25(OH) vit D is a sensitive and specific diagnostic marker of osteoporosis in CLD. More studies are needed to verify GLP-1 role in diagnosis of osteoporosis, the recommended daily dose of vitamin D and calcium and role of GLP-1 against in children with CLD.

Author Contributions

NFE: Contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results, read and approved the final manuscript. OG: Contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results, read and approved the final manuscript. MS: Contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results, read and approved the final manuscript. OE: Contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results, read and approved the final manuscript. D S: Contributed to the design and implementation of the research, aided in choosing the patients, performed the laboratory work and helped shape the research, supervised the findings of this work, discussed the results, read and approved the final manuscript. All authors have read and approved the final manuscript.

FUNDING

Authors declare that there was no extramural funding of the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with the reported study.

References

1. A. Sardar, A. Parkash, A. A. Merchant, B. Qamar, F. Ayub, S. Zehravi, Etiology in Children Presented With Chronic Liver Disease in a Tertiary Care Hospital. *Cureus* **14**, e25570 (2022).
2. K. M. Loomes, C. Spino, N. P. Goodrich, T. N. Hangartner, A. E. Marker, J. E. Heubi, B. M. Kamath, B. L. Shneider, P. Rosenthal, P. M. Hertel, S. J. Karpen, J. P. Molleston, K. F. Murray, K. B. Schwarz, R. H. Squires, J. Teckman, Y. P. Turmelle, E. M. Alonso, A. H. Sherker, J. C. Magee, R. J. Sokol, Childhood Liver Disease Research Network, Bone Density in Children With Chronic Liver Disease Correlates With Growth and Cholestasis. *Hepatol. Baltim. Md* **69**, 245–257 (2019).
3. M. A. Kotb, L. A. Fawaz, R. A. Zeitoun, Y. M. Shaalan, N. Aly, H. Abd El Kader, G. El Tagy, H. Esmat, A. F. Hamza, H. Abd El Baky, Bone demineralization in a cohort of Egyptian pediatric liver transplant recipients: Single center pilot study. *Medicine (Baltimore)* **101**, e31156 (2022).
4. H. L. Ferjani, I. Cherif, D. B. Nessib, D. Kaffel, K. Maatallah, W. Hamdi, Pediatric and adult osteoporosis: a contrasting mirror. *Ann. Pediatr. Endocrinol. Metab.* **29**, 12–18 (2024).
5. J. P. Giblett, S. J. Clarke, D. P. Dutka, S. P. Hoole, Glucagon-Like Peptide-1. *JACC Basic Transl. Sci.* **1**, 267–276 (2016).
6. C. Zhao, J. Liang, Y. Yang, M. Yu, X. Qu, The Impact of Glucagon-Like Peptide-1 on Bone Metabolism and Its Possible Mechanisms. *Front. Endocrinol.* **8**, 98 (2017).
7. T. D. Müller, B. Finan, S. R. Bloom, D. D'Alessio, D. J. Drucker, P. R. Flatt, A. Fritsche, F. Gribble, H. J. Grill, J. F. Habener, J. J. Holst, W. Langhans, J. J. Meier, M. A. Nauck, D. Perez-Tilve, A. Pocai, F. Reimann, D. A. Sandoval, T. W. Schwartz, R. J. Seeley, K. Stemmer, M. Tang-Christensen, S. C. Woods, R. D. DiMarchi, M. H. Tschöp, Glucagon-like peptide 1 (GLP-1). *Mol. Metab.* **30**, 72–130 (2019).
8. World Medical Association, WMA Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subjects (2013). https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/2013/.
9. M. Nouri-Vaskeh, N. Khalili, A. Khalaji, P. Behnam, L. Alizadeh, S. Ebrahimi, N. Gilani, M. Mohammadi, S. A. Madinehzadeh, M. Zarei, Circulating glucagon-like peptide-1 level in patients with liver cirrhosis. *Arch. Physiol. Biochem.* **129**, 373–378 (2023).
10. M. Krishna, Histological Grading and Staging of Chronic Hepatitis. *Clin. Liver Dis.* **17**, 222–226 (2021).
11. D. Madhuchani, S. N. Seneviratne, L. M. Ward, Bone health in childhood and adolescence: an overview on dual-energy X-ray absorptiometry scanning, fracture surveillance and bisphosphonate therapy for low-middle-income countries. *Front. Endocrinol.* **14**, 1082413 (2023).

12. P. Głuszko, E. Sewerynek, W. Misiorowski, J. Konstantynowicz, E. Marcinowska-Suchowierska, T. Blicharski, M. Jabłoński, E. Franek, T. Kostka, M. Jaworski, E. Karczmarewicz, E. Kalinka, K. Księżopolska-Orłowska, A. Więcek, R. S. Lorenc, Guidelines for the diagnosis and management of osteoporosis in Poland. Update 2022. *Endokrynol. Pol.* **74**, 5–15 (2023).
13. J. Kang, H. Gopakumar, S. R. Puli, Prevalence of Osteoporosis in Cirrhosis: A Systematic Review and Meta-Analysis. *Cureus*, doi: 10.7759/cureus.33721 (2023).
14. G. Handzlik-Orlik, M. Holecki, K. Wilczyński, J. Duława, Osteoporosis in liver disease: pathogenesis and management. *Ther. Adv. Endocrinol. Metab.* **7**, 128–135 (2016).
15. National Center for Environmental Health, "Facts About Bone Density (DEXA Scan)" (2024); https://www.cdc.gov/radiation-health/data-research/facts-stats/dexa-scan.html#:~:text=The%20amount%20of%20radiation%20used,developing%20cancer%20later%20in%20life.
16. C. S. Marathe, C. K. Rayner, K. L. Jones, M. Horowitz, Glucagon-like peptides 1 and 2 in health and disease: A review. *Peptides* **44**, 75–86 (2013).
17. I. Daniilopoulou, E. Vlachou, G. I. Lambrou, A. Ntikoudi, E. Dokoutsidou, G. Fasoi, O. Govina, A. Kavga, A. N. Tsartsalis, The Impact of GLP1 Agonists on Bone Metabolism: A Systematic Review. *Med. Kaunas Lith.* **58**, 224 (2022).
18. L. M. Sherief, A. Ali, A. Gaballa, G. M. Abdellatif, N. M. Kamal, M. R. Afify, D. H. Abdelmalek, S. A. El-Emari, A. S. A. Soliman, W. A. Mokhtar, Vitamin D status and healthy Egyptian adolescents: Where do we stand? *Medicine (Baltimore)* **100**, e26661 (2021).
19. Ö. Pazarci, H. O. Dogan, S. Kilinc, Y. Çamurcu, Evaluation of Serum Glucagon-Like Peptide 1 and Vitamin D Levels in Elderly Patients with Bone Fractures. *Med. Princ. Pract. Int. J. Kuwait Univ. Health Sci. Cent.* **29**, 219–224 (2020).
20. A. Nappo, S. Sparano, T. Intemann, Y. A. Kourides, L. Lissner, D. Molnar, L. A. Moreno, V. Pala, I. Sioen, T. Veidebaum, M. Wolters, A. Siani, P. Russo, Dietary calcium intake and adiposity in children and adolescents: Cross-sectional and longitudinal results from IDEFICS/I.Family cohort. *Nutr. Metab. Cardiovasc. Dis.* **29**, 440–449 (2019).