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Original article

Evaluation of Serum Glucagon Like Peptide-2 in Children with Chronic Liver Diseases

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Abstract:

Background: Chronic liver disease (CLD) in children is a significant cause of morbidity and mortality, often leading to complications such as osteoporosis due to nutritional, metabolic, and hormonal factors and intestinal dysbiosis. Glucagon-like peptide-2 (GLP-2), primarily expressed in the ileum, has gained attention for its potential role in bone metabolism. Aim of the study: This study aimed to evaluate serum GLP-2 levels in children with CLD and explore its association with liver disease severity and osteoporosis. **Patients and methods:** A comparative cross-sectional study included 30 pediatric CLD patients and 30 age- and sex-matched apparently healthy controls. Serum GLP-2 levels were measured using an ELISA kit, and bone mineral density (BMD) was assessed using a DEXA scan. Liver fibrosis was scored histologically and liver disease severity was assessed by MELD, PELD, and Child-Pugh scores. **Results:** Serum GLP-2 levels were significantly lower in CLD patients (179.9 ± 91.5 Pg/ml) compared to controls (426.64 ± 133.72 Pg/ml; $P < 0.001$). GLP-2 levels negatively correlated with liver enzyme levels (AST, ALP, GGT; $P < 0.05$) and positively with BMD ($r = 0.414$, $P = 0.023$). The ROC curve revealed a serum GLP-2 cutoff of ≤ 268 Pg/ml, yielding 93.3% sensitivity and 90% specificity for diagnosing CLD. **Conclusion:** Serum GLP-2 may serve as a non-invasive biomarker for liver fibrosis and osteoporosis in pediatric CLD patients. Further researches to confirm its diagnostic utility are needed.

Keywords: Chronic liver disease; Glucagon-like peptide-2; Osteoporosis; Biomarker; Pediatric

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Introduction

Liver cirrhosis and fibrosis are the end results of chronic liver disease (CLD), which can be described as the progressive destruction with regeneration of liver parenchyma that has been present for at least 6 months [1]. Among children and adolescents, CLD is a major cause of illness and death [2].

Up to 40% of patients with liver fibrosis may experience a fracture [3]. Osteoporosis is a systemic illness described by low bone mass and thinning of bone tissues leading to bone fragility and fractures with a negative influence on people's life by restricting mobility and independence [4].

The pathophysiology of CLD-associated osteoporosis is complex. Nutritional, metabolic, and hormonal factors, and intestinal dysbiosis play important roles in the pathogenesis of osteoporosis [5].

Intestinal dysbiosis that occurs in CLD patients mainly due to malnutrition appears to have a significant contribution to increase severity of liver disease and increase bone resorption. A condition called as "leaky gut syndrome" can develop if it causes intestinal inflammation and permeability [6]. Disruption of the intestinal epithelial barrier allows dysbiosis mediators from the gut, like bacterial components or metabolites, to enter the liver. These dysbiosis-derived gastrointestinal mediators contribute to steatosis inflammation; stimulate the immune system and oxidative pathways to promote fibrogenesis. The portal vein collects blood from the mesenteric veins of the intestines and delivers it to the liver providing the organ with 70–75% of its total blood supply. Dysbiosis causes persistent inflammation that induces bone resorption by activating immune cells [7, 8].

The terminal ileum is the primary site of Glucagon like Peptide-2 (GLP-2) expression after eating [9]. From the stomach to the colon, the GI tract is GLP-2's primary site of action. GLP-2 may have some influence on the regulation of function in the digestive tract as it has been shown to increase epithelial proliferation, inhibit apoptosis, enhance barrier function and increase digestion, absorption and blood flow [10].

Recent years have seen a rise in studies examining GLP-2 and its role in osteoporosis and its effects on bone tissue along with intermediate metabolism pathways. In some osteoblast cell lines, it has been hypothesized that a peptide receptor comparable to glucagon-2 is present. GLP-2 is able to trigger the formation of osteoblasts from bone marrow cells. It also promotes osteoblast activity, resulting in an increased bone mineralization and the synthesis of organic bone components [11].

Aim of the study:

We decided to conduct this study to identify the association of serum level of GLP-2 and pediatric CLDs and the association of GLP-2 and osteoporosis.

Type of Study: A comparative cross-sectional study.

Subjects and Methods:

Subjects:

The study included 30 patients of CLD selected from the Pediatric Hepatology Clinic at Benha University Hospital between September 2021 and September 2022. Both sexes were included, with ages ranging from 8 months to 18 years. Persons with CLD due to cholestatic liver disease, metabolic liver disorders, autoimmune hepatitis, or chronic hepatitis B or C were included. All the indicatives of chronicity of liver disease were established by clinical, laboratory, as well as histopathological examinations.

Inclusion Criteria:

- Thirty children with CLD were included in this study. CLD means physical stigmata such as hepatosplenomegaly, spider telangiectasia, and clubbing lasting for more than 6 months **[12]**. Children diagnosed with cholestatic liver diseases, metabolic liver disorders, autoimmune

hepatitis or chronic hepatitis B and C were eligible for inclusion in the study.

Exclusion criteria:

- Patients with CLD who also suffered from other conditions (such as cardiovascular, renal, or central nervous system dysfunction).

Between September 2021 and September 2022, thirty apparently healthy, age and sex matched, children were enrolled in the study as a control group. They were those who attended at the pediatric clinic for regular checkup, either for sports physicals or for school physicals.

An informed consent was obtained from the parents (or legal guardian) of all the children included in the study. The study started after receiving approval from the Research Ethics Committee, Faculty of Medicine, Benha University {M.S. 22.8.2021}.

Methods:

Laboratory evaluation of hepatic function by measuring blood counts, liver function tests (Alkaline Phosphatase, Alanine Aminotransferase, Aspartate Aminotransferase, Gamma-Glutamyl Transpeptidase, Total and Direct Bilirubin and Serum Albumin), clotting time (Complete Blood Count, PT, and aPTT), as well as a full clinical evaluation were all done for all the enrolled children. The histopathological analysis of the liver biopsies was done according to the Ishak score.

Osteoprotic profile was studied by serum calcium, serum 25(OH) Vitamin D and bone mineral density (BMD) using dual energy x-ray absorptiometry (DEXA).

Serum GLP-2 was measured in both CLD patients and apparently healthy controls. The relation between GLP-2 and both severity of CLD and osteoprotic changes, was identified.

Human Glucagon-like peptide 2 ELISA kit (Sunred biotechnology company; Catalog No. 201-12-5849) was used to measure serum GLP-2 concentration in accordance with the manufacturer's recommendations.

Menghini aspiration needles (Hepafix Luer Lock Braun Melsungen AG, Melsungen, Germany) were used for ultrasound-guided liver biopsies in the CLD group. A core was extracted from the liver with at least 11 portal tracts, settled in formalin, also sections were stained with both eosin and hematoxylin to evaluate the staging of liver fibrosis using the Ishak scoring system and histological activity. F0 (no fibrosis), F(1-2/6) (Fibrous expansion of some or most portal areas, with or with no shorter fibrous septa), F(3-4/6) (Fibrous expansion of most portal areas with occasional or marked bridging (portal-portal as well as portal-central(4-5/6), F(5-6/6) (Marked bridging (portal-portal, and/or portal-central) with occasional nodules (incomplete cirrhosis)or cirrhosis). Mason-Trichrome stain, Perls' Prussian blue stain, along with periodic acid chief stain were used for determining fibrosis severity, iron deposition, and alpha 1 anti-trypsin deficiency, respectively.

The severity of liver disease was measured in children and adolescents using the model for end-stage liver disease (MELD) score, the child-related end-stage liver disease (PELD) score and the Child-Pugh score for those aged 12 as well as up, 6-11 years old, and 1-11 years old, respectively.

Statistical analysis:

The results were gathered and tabulated using SPSS v. 16 (Spss Inc., Chicago, IL, USA). Qualitative data were compared using the chi-square (χ^2) test; numbers were expressed as percentages. The quantitative data were presented as mean ± SD. Comparison between two or more groups was done using Student (t) & ANOVA (F) tests. Variable correlations were assessed using Pearson's correlation coefficient (r). Receiver-operating characteristic (ROC) curve analysis was utilized to find serum GLP-2 cut-off values for both early and advanced fibrosis diagnosis with optimal sensitivity and specificity. Multivariate analysis predicted fibrosis scores. The results were deemed

significant at a P value of 0.05 with a 95 percent confidence interval.

Results

Table (1): Comparison between serum GLP-2 and degree of fibrosis and degree of histological activity index

Variables		Serum glucagon-like peptide-2 (GLP-2) (Pg/ml)		f-test	p-value
		Mean ± SD	Range		
Degree of fibrosis	Mild	8230.50± 1385.22	748 - 5720	5.31	0.000
	Moderate	3182.50± 1641.36	950 - 7410		
	Severe	1799.32 ± 977.33	7251 - 9210		
Histological activity index	Minimal	6010.33± 3064.83	748 - 3053	35.007	0.000
	Mild	7251	941 – 5410		
	Moderate	2251.00± 1572.76	7251		
	Severe	1666.34 ± 525.12	3101 – 9210		

GLP-2= Glucagon Like Peptide-2.

Table (1) shows that there was a statistically significant decrease in GLP-2 with increase in degree of fibrosis and histological activity index in the hepatic group.

Table (2) shows that there was a statistically significant positive correlation between bone marrow density and serum GLP-2 (Pg/ml) in the hepatic group.

Table (2): Comparison between the two studied groups according to BMD and its Z-score

Dexa Scan	Hepatic group (n = 30)	Control group (n = 30)	U	P
BMD[Lumbar spine](g/cm^2)				
Range	0.07 – 0.18	0.17 – 0.32		
Mean ± SD.	0.14 ± 0.03	0.26 ± 0.05	6.50	§
Median (IQR)	0.14 (0.13 – 0.16)	0.24 (0.22 – 0.32)		
Z-score				
Range	-3.50 – -0.40	-1.0 – 1.0		
Mean ± SD.	-2.04 ± 0.88	0.13 ± 0.82	24.0	§
Median (IQR)	-2.50 (-2.60 – -1.20)	0.0(-1.0 – 1.0)		

§ = P value <0.001, BMD= Bone mineral density.

Table (3) shows that there was statistically significant negative correlation between bone mineral density and severity of liver diseases as the increase of osteoporosis is associated with an increase in severity of liver diseases in the hepatic group.

Table (4) shows that there was a statistically significant negative correlation between AST, ALP and GGT and serum GLP-2 in the hepatic group. While there was a statistically significant positive correlation between BMI centile, HGB, T.ca, I.ca and Vit D and serum GLP-2 in hepatic group.

The best cut-off serum GLP-2 for diagnosis of CLD in children was ≤ 268 Pg/ml with a sensitivity of 93.33%, a specificity of 90.0%, a positive predictive value 90.3%, a negative predictive value 93.1%, and a fair area under the ROC curve (AUC) of 914.0 as shown in **Figure 1**.

Table (3): Correlation between severity of osteoporosis and severity of liver diseases in hepatic group

Variables	Bone mineral density [Lumbar spine] (g/cm2)		Z-score	
	rs	p	rs	p
PELD score	-0.127	0.028	-0.593	0.012
MELD score	-0.545	0.044	-0.571	0.042
Child Pugh score	-0.158	0.040	-0.528	0.003

BMD= Bone mineral density, MELD= Model for End-stage Liver Disease, PELD= Paediatric End-stage Liver Disease.

Table (4): Correlation between serum GLP-2 (Pg/ml) and severity of osteoporosis and different parameters in the hepatic group.

Variables	Serum GLP-2 (Pg/ml	
	rs	P
BMD	0.414	0.023*
Z score	0.037	0.845*
BMI centile	0.366	0.046*
AST	-0.374	0.042*
ALP	-0.467	0.009*
GGT	-0.411	0.024*
HGB	0.390	0.033*
T.Ca	0.606	<0.001*
I.Ca	0.473	0.008*
25 (OH)Vit D	0.552	0.002*
PELD score	-0.205	0.429*

GLP-2=Glucagon Like Peptide-2, BMD= Bone mineral density, BMI= Body Mass Index, AST= Aspartate Aminotransferase, ALP= Alkaline Phosphatase, GGT= Gamma Glutamyl Transferase, HGB= Hemoglobin, T. ca= Total calcium, T. ca= Total calcium, 25(OH)Vit D= 25 hydroxy Vitamin D, PELD= Paediatric End-stage Liver Disease.

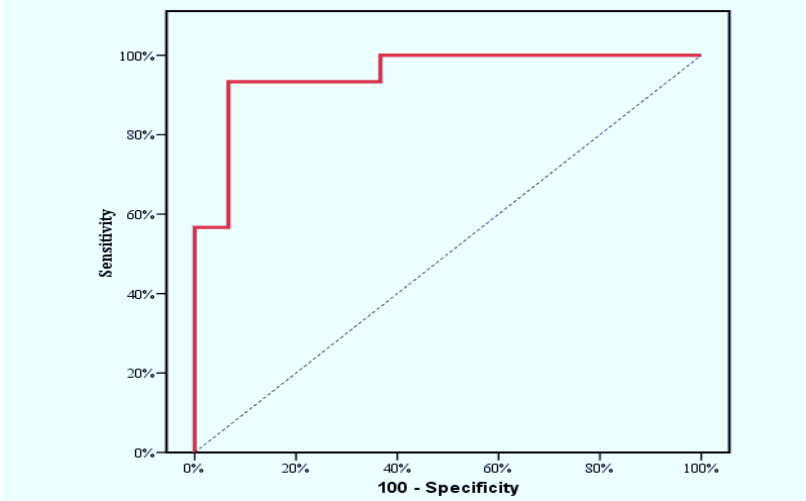

Figure (1): ROC curve analysis of serum GLP-2 in diagnosing pediatric CLD.

Discussion

According to the findings of the current investigation, serum concentration of Glucagon-like Peptide-2 was considerably lower in those with CLD than it was in healthy children. In addition, serum GLP-2 level declined significantly, as the degree of fibrosis progressed. The histological activity index was also reduced significantly when there was a high Child-Pugh score, PELD score, or MELD score. Our study is the first study to assess the role of serum GLP-2 in CLD in children.

GLP-2 exhibit enlarged liver macrophage activation & bacterial translocation in addition to heightened biochemical indicators of cholestasis, including bile acids, already at baseline [13, 14]. Hepatic stellate cells (HSCs) are positioned in close proximity to von Kupffer cells (vKCs), the liver's resident macrophages, which regulate HSC activation. Hepatic stellate cells contribute significantly to hepatic fibrogenesis and are activated by vKCs, liver damage, and inflammation. By producing more alpha-smooth muscle actin (α-SMA) and other extracellular matrix (ECM) proteins, when triggered, these cells take on a myofibroblast-like character and become more contractile. Many profibrotic cytokines, containing transforming growth factor-β and platelet-derived growth factor-BB, are secreted by vKCs during fibrogenesis in mice [15].

In the current trial, serum GLP-2 had a statistically significant positive association with bone mineral density, meaning that GLP-2 level decreases with progression of osteoporosis in CLD children.

This agrees with a study [12] which reported that, total hip bone mineral density improved after GLP-2 therapy. Moreover, a study [15] examined the impact of parenteral administration of GIP along with GLP-2 on bone turnover processes in healthy volunteers, demonstrating that they decrease bone resorption. GIP is a gut hormone that is secreted in tandem with GLP-2 after the ingestion of glucose, fat, protein, as well as fructose. Also, Glucagon-like peptide-2 was reported to be a safe medication that dramatically reduced bone resorption when given to healthy postmenopausal women for two weeks [16].

GLP-2's impacts on bone tissue in humans have been researched extensively. Some cell lines of osteoblasts have shown enhanced osteocalcin production in response to GLP2, suggesting the presence of a peptide receptor similar to glucagon-2 [17] the underlying molecular mechanisms of its action have yet to be understood [18].

GLP-2 stimulates osteoblasts, which are bone-forming cells and inhibits osteoclasts, which are bone-resorbing cells. Because of this, there is less of bone resorption and a rise in bone formation, which can ultimately improve bone density and strength [19]. So, the statistically significant positive relationship amongst GLP-2 & BMD, found in our study in CLD children, can emphasize that GLP-2 decrease in our hepatic children has a determinate effect on the liver and its function and subsequently causing osteoporosis in those CLD patients by nutritional, metabolic and hormonal factors and intestinal dysbiosis owing to loss of liver function.

In this study, there was a statistically significant negative association between AST, ALP & GGT and serum GLP-2 in the hepatic group. While there was a statistically positive connection etween BMI centile, HGB, T.ca, I.ca & Vitamin D and serum GLP-2 in the hepatic group. In this study, the diagnostic performance of GLP-2 levels in CLD directed that at cut off value ≤268 (pg/mL), it had a sensitivity of 93.33% and a specificity of 90.0% with a fair area under the ROC curve of 419.0. The PPV was 90.3%, while the NPV was 93.1%. There are no studies about GLP-2

in children with CLD, so we don`t have any comparable cut off values.

The present research had some limitations, including the lack of serial samples that would have allowed for tracking the examined marker as well as the course of the disease & its response to treatment.

Conclusions

Our findings lead us to the conclusion that serum GLP-2 could be one of the less invasive methods to be used for diagnosing & staging hepatic fibrosis in Egyptian children suffering from chronic liver illnesses. It is possible that the findings of this study need to be confirmed by additional research conducted on a larger scale. There is a possibility that additional research may be carried out to investigate the function of GLP-2 in monitoring children who have chronic liver fibrosis as well as their reactions to treatment.

Conflict of interest

None of the contributors declared any conflict of interest

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