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Original article

Evaluation of Serum Golgi Protein 73 as Diagnostic Value of Liver Diseases in Children

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Abstract:

Background: Children with liver diseases often present late due to nonspecific symptoms. Reliable non-invasive biomarkers are needed to improve early diagnosis and staging. Aim: To evaluate the role of serum Golgi protein 73 (GP73) as a diagnostic biomarker for chronic liver diseases and predictor of fibrosis severity in children. **Patients and Methods:** This case-control study included one hundred children: fifty patients with chronic liver diseases (autoimmune hepatitis, chronic hepatitis B/C, metabolic disorders) and fifty healthy controls. Patients underwent clinical assessment, laboratory tests (liver enzymes, coagulation profile, fibrosis indices), and liver biopsy to assess fibrosis by Metavir scoring. Serum GP73 levels were measured by ELISA. ROC analysis assessed diagnostic accuracy. **Results:** GP73 levels were significantly higher in patients (median: 630.3 ng/ml) than controls (median: 337.1 ng/ml; $p<0.001$). GP73 correlated positively with liver span, AST, ALP, APRI, FIB-4, MELD, Child-Pugh scores, and fibrosis stage ($p<0.05$). ROC analysis showed good diagnostic accuracy of GP73 for chronic liver disease (AUC=0.771) and for predicting higher fibrosis (AUC=0.826). Multivariate regression confirmed GP73 as an independent predictor of liver involvement. **Conclusion:** Serum GP73 is a promising non-invasive biomarker for diagnosing pediatric chronic liver diseases and estimating fibrosis severity, potentially reducing need for biopsy.

Keywords: GP73; children; chronic liver disease; biomarker; fibrosis.

Introduction

The spectrum of liver illness is extensive, with several underlying conditions exhibiting both chronic and acute presentations. The primary pathogenetic pathways are attributed to autoimmune illness, viral infection, and toxic insult [(1)]. Hepatic illness is responsible for over two million fatalities annually throughout the globe, with one million attributed to cirrhosis complications and one million to hepatocellular cancer and viral hepatitis [(2)]. Chronic liver diseases (CLD) denote conditions of the liver characterized by a progressive cycle of destruction and regeneration of hepatic tissue, resulting in fibrosis, cirrhosis, and liver insufficiency [(3)]. In children, liver illnesses are frequently discovered late, largely because early symptoms are subtle and nonspecific, such as poor appetite, abdominal pain, or fatigue [(4)].

Golgi protein 73 (GP73) is a transmembrane protein located in the Golgi apparatus, known by other names such as Golgi phosphoprotein II or Golgi membrane protein I [(5)]. The N-terminal part of GP73 can be cleaved by a proprotein convertase, allowing it to be secreted into the bloodstream and measured in serum [(6)]. Recent evidence suggests that elevated serum GP73 levels correlate with hepatic fibrosis and may serve as a non-invasive biomarker for chronic liver diseases, potentially reducing reliance on invasive biopsy.

Compared to other common biomarkers—such as alpha-fetoprotein (AFP), which mainly indicates malignancy; liver enzymes like ALT and AST, which reflect active hepatocellular injury; and composite indices like FibroTest or APRI, which estimate fibrosis—GP73 shows promise in directly reflecting fibrotic remodeling and structural changes [(6)].

Definitive diagnosis of CLD usually requires liver biopsy, which is invasive. Therefore, there is clinical interest in reliable, non-invasive markers that can aid diagnosis and staging [(7)].

This study aimed to evaluate serum GP73 as a diagnostic biomarker specifically for chronic pediatric liver diseases (e.g., chronic hepatitis B/C, autoimmune hepatitis, metabolic liver disorders) and to assess its ability to predict fibrosis severity.

Patients and methods

This case-control study included one hundred children at Pediatric hepatology unit in Benha University Hospitals, Benha, Egypt, for one year from March 2022 to March 2023.

Participants were divided into two groups: Cases group (Num.=50) and control group (Num.=50).

Inclusion criteria: All children <18 years with chronic liver diseases: chronic hepatitis B/C, autoimmune hepatitis, metabolic liver disorders. Acute self-limited hepatitis A was excluded as it does not cause chronic disease." Controls were age- and sex-matched healthy children recruited from general pediatric outpatient clinics after excluding any hepatic, chronic illness, or systemic disease by history, examination, and routine labs."

Methods

All patients were subjected to the following:

Complete History Taking: Comprehensive patient data included demographics (gender, age, residence), details of the current liver condition (symptoms, duration, progression), past medical history (cerebral palsy, malabsorption, renal or liver disease, endocrine disorders, fractures), and family history (parental consanguinity and inherited or metabolic disorders).

Diagnosis: Diagnosis of chronic liver diseases was based on clinical, laboratory, imaging findings and confirmed by liver biopsy using Metavir score."

Clinical Examination

Anthropometrics: Weight, height, BMI; z-scores calculated via WHO standards (AnthroPlus software) and **clinical Signs**: Jaundice, abdominal distension/pain, lower

limb edema, melena, bleeding (hematuria, epistaxis).

Abdominal Exam

Inspection: Abdominal veins, contour, localized swelling, **palpation**: Liver (span, edge, consistency) and spleen (size, tenderness) and **Percussion**: Ascites, liver upper border, and other system examination.

Laboratory Investigations

- Laboratory tests included liver enzymes, coagulation profile, autoantibodies, and metabolic markers."
- Biochemical liver function tests were assessed by DIALAB, 13771103, Thermo company, USA.
- Coagulation Function (APT and INR): using Automated blood coagulation analyzer CS-1600, SN 12058, Sysmex Corporation, Kobe, Japan.

Assessment of Golgi protein 73 level

Serum GP73 levels were measured using a Human GP73 ELISA Kit (Sun Red Biotechnology, Catalog Number. 201-12-1433), By TECAN Infinite F50 ELISA reader, Singapore, S.N. 1306007553, following a double-antibody sandwich protocol. After coagulating serum at room temperature (10–20 min) and centrifuging (2000–3000 rpm, 20 min), samples were incubated in pre-coated monoclonal antibody wells with biotinylated GP73 antibodies and streptavidin-HRP complexes. Chromogen substrates (A/B) induced a color shift to yellow upon acid addition, with optical density (OD450 nm) measured within 10 minutes. A standard curve (OD vs. concentration) was plotted, and sample GP73 levels were calculated via linear regression. Assay steps included 37°C incubations (60 min for immune complexes, 10 min for chromogen reaction), automated washing, and strict adherence to reagent storage (2–8°C).

Indices of Liver Fibrosis

Assessment of pediatric end stage liver disease (PELD) score for CLD cases < twelve years old PELD score utilizes the case's values for serum albumin, serum bilirubin, the international normalized ratio (INR), whether the case has growth failure (below -2 standard deviation) and whether the case is below one year old, to expect survival. It is determined regarding the following formula.

PELD = 4.80[serum total bilirubin (mg/dL)] + 18.57[INR] - 6.87 [albumin (g/dL)] + 4.36 (zero to 10 were considered moderate liver disease. Results >10 were considered sever liver disease [(8)].

Assessments of model for end-stage liver disease score for CLD patients > twelve years old. MELD utilizes the case's blood bilirubin, serum creatinine, and INR for prothrombin time to forecast survival. The calculation is based on the following formula.

MELD = 3.78x [serum total bilirubin (mg/dL)] + 11.2x [international normalized ratio] + 9.57x [serum creatinine (mg/dL)] + 6.43 MELD Na = MELD - Serum Na - [0.025 * MELD * (140 - Serum Na)] + 140. Results from zero to ten were considered mild liver disease. Results from >10 to 20 were considered moderate liver disease. Results >20 were considered sever liver disease [(9)].

Aspartate aminotransferase to platelet ratio index (APRI), the APRI were calculated as follows: APRI = (AST/upper limit of a normal range of AST) × 100)/platelet count (109/L) [(10)].

Fibrosis-4 (FIB-4) = [age (years) × AST (IU/L)]/ [platelets count (109/L) × √ALT (IU/L)] [(11)].

Child-Pugh Score:

The Child-Pugh grading system was devised by Turcotte and Child in 1964 to assist in the identification of patients suitable for elective surgery for portal decompression, categorizing cases into 3 groups: A - good liver function, B - moderately impaired liver function, and C - severe liver dysfunction. Their first scoring system included five laboratory and clinical criteria to classify cases: serum albumin, neurological disease, serum bilirubin, ascites, and clinical nutritional status [(12)].

Pelvi-abdominal Ultrasonography: For evaluation of spleen span, texture, hepatic span, and presence of ascites. Normal ranges of liver and spleen are shown in table I &II.

Liver Biopsy for case group only

A Menghini aspiration needle (Hepafix Luer Lock, Braun Melsungen AG, Melsungen, Germany) was used to conduct ultrasound-guided liver biopsies on all study participants. Each biopsy procured an adequate core, encompassing at least eleven portal tracts. The retrieved samples were promptly preserved in formalin, then embedded within paraffin for subsequent examination. Sections measuring five micrometers thick were meticulously prepared, affixed to glass slides, and subjected to hematoxylin and eosin staining. This process was undertaken to evaluate the histological activity of hepatitis, employing the Metavir scoring system for systematic assessment [(13)].

Additionally, Masson's trichrome staining was employed to evaluate the stage of fibrosis. Iron deposition was identified using Prussian blue stain, and PAS staining was conducted to exclude alpha-1 antitrypsin (A1AT) deficiency.

Sample size clarification.

"Sample size was initially estimated as 55 based on effect size=0.5, α=0.05, power=80% (G*Power 3.1.9.2). To improve validity, it was increased to 100 participants (50 patients, 50 controls)."

Ethical consideration

The research complies with the Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. The whole research design has been approved by the local ethics committee, Faculty of Medicine, Benha University. Confidentiality and personal privacy have been respected at all the levels of the research. Guardians were free to withdraw from the research at any time without any consequences. Gathered data were not and will not be utilized for any other purpose. Approval NO {M.S.24.2.2022}.

Statistical analysis and data interpretation

The data have been input into the analyzed and computer with IBM SPSS software (version 22.0). Qualitative data have been characterized by utilizing numbers and percentages. Quantitative data have been characterized by median (maximum and minimum) for non-parametric data and mean, ±SD for parametric data, following normality assessment by the Kolmogorov-Smirnov test. The significance of the outcomes attained has been assessed at the 0.05 level. These tests were used: The Chi-Square test is utilized for the comparative analysis of two or more groups. The Monte Carlo test serves as a correction for the Chi-Square test when over twenty-five percent of cells have counts below five in tables more than 2x2. The Fisher Exact test is utilized as a correction for the Chi-Square test when over twenty percent of cells have counts below five in 2x2 tables. The student's t-test is applied to compare 2 independent groups, while the Mann-Whitney U test is used for comparing 2 independent groups as well. Spearman's correlation assesses the association among two variables with non-parametric quantitative data. **The results were explained as follows:** if the p-value < 0.05 has been insignificant, the p-value is below or equal to 0.05 was a significant, and significant if the p-value is below 0.001.

Results

cases showed significantly lower median weight (15 kg vs. 25 kg), mean height (0.95 ± 0.2 m vs. 1.1 ± 0.2 m), weight centiles (25 vs. 60), and height centiles (25 vs. 70) than controls ($P < 0.001$ for all). BMI was also reduced in patients (median: 16.5 vs. 19.3, *P = 0.001*). Age, sex, and residence showed insignificant variances ($P > 0.05$). Consanguinity was reported in 32% of cases and the median of disease duration among patients was 14 months (range 12–18). **(Table 1).**

The biopsy revealed lymphocytes as the most common type of cell, followed by inflammatory and plasma cells. Eosinophils were detected in 10% of patients. Liver biopsy revealed varying degrees of fibrosis, with patients classified as F1, F2, F3, or F4. **(Table 2)**

GP73 levels were markedly elevated in patients (median: 630.3 ng/ml; range: 96.9–1366) compared to controls (median: 337.1 ng/ml; range: 32–724.3) (P below 0.001). WBC count (P = 0.651) showed insignificant variances between the groups. **(Table 3)**

There were no significant differences in GP73 levels across F1, F2, F3, and F4 stages, with a P-value of 0.109. The mean ± SD GP73 levels were 443.47 ± 264.69 for F1, 508.13 ± 329.75 for F2, 539.38 ± 491.83 for F3, and 939.15 ± 408.95 for F4. The median GP73 levels (Min-Max) were 413 (96.9–1199) for F1, 448.2 (121.7–1124) for F2, 433.2 (107.7–1266) for F3, and 1013 (159.1–1366) for F4**(Table 4**).

There was positive correlation between GP73 levels and several factors such as liver span, AST levels, ALP levels, APRI score, FIB-4 score, Pediatric MELD score, CTP score, and degree of fibrosis. However, GP73 levels did not show significant correlations with age, weight, height, BMI, age of onset of liver disease, spleen size, creatinine, total bilirubin, ALT, GGT, PT, APTT, INR, serum total protein, serum albumin, hemoglobin, WBC count, platelet count, PELD, and histological activity index p below 0.05. **(Table 5).**

Among the variables included in the multivariate logistic regression analysis, only serum GP73 level showed a statistically significant association with hepatic affection (OR = 1.004, 95% CI: 1.002–1.006, p < 0.001). Age, sex, residence, and BMI were not significant predictors. **(Table 6)**

The GP73 showed good diagnostic accuracy with an AUC of 0.771, a ninety-five percent confidence interval of 0.680–0.862, and a statistically significant positive and negative predictive value. **(Table 7).**

Table (1): General features of the examined groups

		Patients (num. = fifty)	**Controls (num. = fifty)**	**P-value**
Age (years)	Median (range)	4 (0.3 - 13)	5 (1 - 9)	0.322
Sex				
Males	n (%)	11 (22)	15 (30)	0.362
Females	n (%)	39 (78)	35 (70)	
Residence				
Rural	n (%)	19 (38)	21 (42)	0.683
Urban	n (%)	31 (62)	29 (58)	
Consanguinity	n (%)	16 (32%)	-	-
Weight (kg)	Median (range)	15 (3.75 - 40)	25 (9 - 42)	**<0.001***
Height (m)	Mean ±SD	0.95 ±0.2	1.1 ±0.2	**<0.001***
Weight centile	Median (range)	25 (3 - 99)	60 (15 - 97)	**<0.001***
Height centile	Median (range)	25 (2 - 99)	70 (3 - 97)	**<0.001***
BMI	Median (range)	16.5 (11.8 - 50.7)	19.3 (14.7 - 24.6)	**0.001***
Disease duration (months)	Median (range)	14.0 (12.0 – 18.0)	-	-

kg: Kilogram; SD: Standard deviation; BMI: Body mass index; m: Meter.

Table (2): Liver biopsy of hepatic patients

Liver biopsy finding		**Patients N= 50**	
		N	**%**
Type of cells	Eosinophils	5	10%
	Lymphocytes	17	34%
	Mononuclear inflammatory cells	14	28%
	Plasma cells	14	28%
Degree of fibrosis	F1	18	36%
	F2	21	42%
	F3	5	10%
	F4	6	12%
Histological activity index	A0	25	50%
	A1	10	20%
	A2	11	22%
	A3	4	8%

Table (3): GP73 Level of the examined groups.

		Patients (num. = 50)	**Controls (num. = 50)**	**P-value**
GP73 Level	Mean ±SD	630.3 3.172	337.1 173.01	**<0.001***
	Median (range)	630.3 (96.9 - 1366)	337.1 (32 - 724.3)	

Table (4): GP73 Level according to different degrees of fibrosis.

GP73 Level	F1	F2	F3	F4	P-value
Mean±SD	443.47±264.69	508.13±329.75	539.38± 491.83	939.15± 408.95	0.109
Median (Min-Max)	413(96.9-1199)	448.2(121.7-1124)	433.2(107.7-1266)	1013(159.1-1366)	

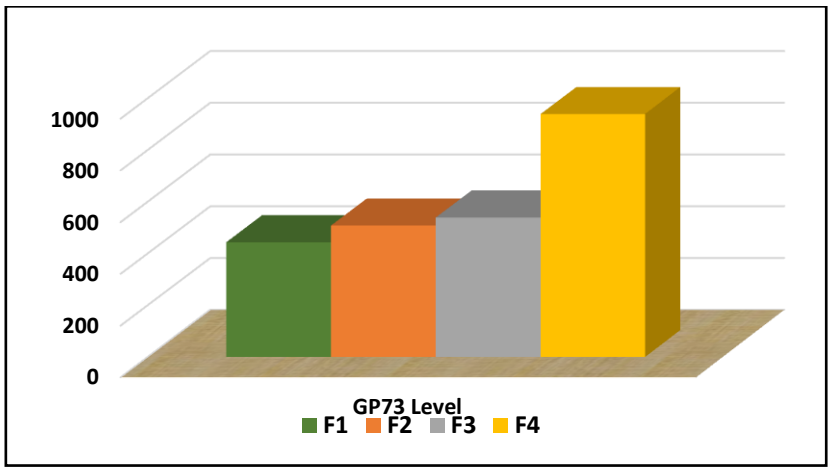

Figure (1): GP73 Level according to different degrees of fibrosis.

Table (5): Association among GP73 Level and other parameters in hepatic cases

	GP73 Level	
	r	**P**
Age (years)	0.015	0.92
Weight (kg)	-0.023	0.872
Height (m)	0.003	0.984
BMI	-0.084	0.561
Age of onset of liver disease	-0.024	0.87
Liver span (cm)	.292	**0.04***
Spleen size (cm)	0.099	0.494
Creatinine (mg/dl)	0.068	0.637
Total bilirubin	0.051	0.724
Direct bilirubin	0.144	0.318
AST (U/L)	.430	**0.002***
ALT (U/L)	0.266	0.062
ALP (U/L)	0.359	**0.01***
GGT (U/L)	0.193	0.179
PT (sec)	-0.007	0.962
APTT (Sec)	-0.009	0.951
INR	0.02	0.892
Serum total protein (g/dl)	0.174	0.227
Serum Albumin (g/dl)	0.013	0.93
Hb (g/dl)	0.037	0.797
WBCs (×103/µl)	-0.117	0.419
PLTs (×103/µl)	-0.009	0.95
APRI	0.410	**0.003***
FIB-4	0.324	**0.022***
Pediatric MELD	0.354	**0.012***
PELD	0.15	0.27
CTP score	.338	**0.016***
Degree of fibrosis	0.376	**0.007***
Histological activity index	0.157	0.277

GP73: Golgi protein 73; r: Correlation coefficient; P: P-value; ALP: Alkaline phosphatase; PT: Prothrombin time; GGT: Gamma-glutamyl transferase; APTT: Activated partial thromboplastin time; INR: International normalized ratio; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Hb: Hemoglobin; PLTs: Platelets; APRI: AST to platelet ratio index; WBCs: White blood cells; FIB-4: Fibrosis-4 index; MELD: Model for end-stage liver disease; CTP: Child-Turcotte-Pugh.

Table (6): Multivariate logistic regression analysis to predict hepatic affection.

	OR (ninety-five percent CI)	**P-value**
Age (years)	0.988 (0.834 - 1.169)	0.884
Sex	1.323 (0.467 - 3.75)	0.599
Residence	1.171 (0.46 - 2.986)	0.74
BMI	0.967 (0.883 - 1.059)	0.469
GP73 Level	1.004 (1.002 - 1.006)	**<0.001**

OR: Odds ratio; CI: Confidence interval.

Table (7): Performance of GP73 Level to diagnose liver fibrosis in chronic liver disease.

ROC characteristics	
AUC	0.771
Ninety-five percent CI	0.680 – 0.862
Best cutoff	> 429.3
Sensitivity	68%
Specificity	64%
PPV	65.4%
NPV	66.7%
P-value	**<0.001***
ROC characteristics	
AUC	0.826
Ninety-five CI	0.687 – 0.965
Best cutoff	> 777.9
Sensitivity	87.5%
Specificity	71.4%
PPV	36.8%
NPV	96.8%
P-value	**0.004***

NPV: Negative Predictive Value; AUC: Area Under the Curve; PPV: Positive Predictive Value Sensitivity: True positive rate; Specificity: True negative rate.

Discussion

Monitoring GP73 expression in children with chronic liver disease may reflect underlying hepatic abnormalities and regenerative capacity [(14)]. Our study showed that there were no significant differences in age, sex, or residence between the groups. However, patients showed significantly lower weight, height, BMI, and growth percentiles, indicating growth impairment associated with chronic liver disease.

This aligns with Qian et al. [(15)], who also found BMI differences but no significant differences in age or sex.

We observed significantly higher serum GP73 levels in patients than controls, in agreement with Liu et al. [(6).] Additionally, fibrosis assessment by liver biopsy showed varying degrees among patients, and clinical severity was reflected in MELD, PELD, and Child-Pugh scores, like findings by Panezai et al. [(16)] and Rahimi et al. [(17)].

Importantly, GP73 levels correlated positively with liver span, AST, ALP, APRI, FIB-4, MELD, Child-Pugh scores, and fibrosis stage, supporting its association with disease severity and fibrosis [(6,18)]. Multivariate analysis confirmed GP73 as an independent predictor of both liver involvement and advanced fibrosis.

Beyond its statistical significance, GP73 has important clinical implications: as a non-invasive biomarker, it could help identify children at risk of advanced fibrosis, potentially reducing the need for routine liver biopsy. Compared to conventional markers like ALT and AST, which reflect inflammation but not fibrosis, or indices like APRI and FIB-4, which can be limited by age-related variation in children, GP73 directly reflects fibrotic remodeling **[(6)]**. Moreover, while FibroScan is useful, it may be limited by technical challenges in small children; GP73 measurement via ELISA is simpler and widely accessible. Overall, GP73 shows promise as a complementary tool alongside existing markers to improve early detection and staging of pediatric chronic liver disease.GP73 may be better than ALT, AST, APRI, or FibroScan because it detects liver fibrosis earlier and more specifically, is easy to measure via blood test, and complements other tests to improve diagnosis accuracy **[(19)]**.

Limitations

Single-center study; small sample; heterogeneous etiologies; lack of long-

term follow-up. Further multicenter studies with larger cohorts are recommended.

Conclusion

GP73 is a promising non-invasive biomarker that correlates strongly with liver fibrosis severity and disease progression in children with chronic liver disease. It offers advantages over conventional markers like ALT, AST, APRI, and FibroScan by detecting fibrosis earlier and more specifically, while being easy to measure through a simple blood test. Incorporating GP73 alongside existing diagnostic tools may improve early detection, accurate staging, and management of pediatric liver disease, potentially reducing the need for invasive procedures such as liver biopsy.

Conflict of interest

None declared any conflict of interest.

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