Journal of
Gastroenterology and Hepatology Research

Online Submissions: http: //www.ghrnet.org/index./joghr/
doi: 10.17554/j.issn.2224-3992.2021.10.1030

Journal of GHR 2021 December 21; **10(6)**: 3619-3624
ISSN 2224-3992 (print) ISSN 2224-6509 (online)

ORIGINAL ARTICLE

Clinical Value of Serum Glypican-3 in Children With Biliary Atresia

Ola Galal Ali Behairy[1], Amira Mohamed Nour Eldein Abdelrahman[2], Shorouk Zaky Abdelaziz Abdelshafy[3], Asmaa Ali Mahrous[4]

1 Pediatrics department, Faculty of Medicine, Benha University, Egypt;
2 Clinical and chemical pathology department, Faculty of Medicine, Benha University, Eygpt;
3 Diagnostic & Interventional Radiology, Faculty of Medicine, Benha University, Eygpt;
4 M.B.B.Ch., Faculty of Medicine, Benha University, Egypt.

Conflict-of-interest statement: The authors declare that there is no conflict of interest regarding the publication of this paper.

Correspondence to: Ola Galal Ali Behairy, Faculty of Medicine-Benha University, Benha, Qualiopia, Egypt.
Telephone: +01011655885
Email: olaped99@yahoo.com

Received: October 1, 2021
Revised: October 19, 2021
Accepted: October 23, 2021
Published online: December 21, 2021

ABSTRACT

BACKGROUND: Glypican-3 (GPC-3) is a key regulator of a variety of physiological processes, including cellular growth, differentiation, and cell proliferation, and morphogenesis, particularly in hepatocytes. This study aimed to assess serum GPC-3 levels in children with biliary atresia (BA) and its correlation with clinical parameters.

METHODS: In this case-control study 50 children, with biliary atresia, and 50 healthy children as controls. All children were diagnosed with biliary atresia based on clinical, laboratory, and histological findings of liver biopsy. All children had their medical histories were taken, complete clinical examination, and serum GPC-3 levels by ELISA.

RESULTS: Glypican 3 was statistically higher in the biliary atresia group (7.26 ± 3.48 ng/ml) than the control group (1.7 ± 0.52 ng/ml), $p < 0.001$. Also, it was higher in BA with jaundice (9.1 ± 2.66 ng/ml) than BA without jaundice (3.6 ± 1.2 ng/ml) and controls, $p < 0.001$. Moreover, GPC-3 was higher in BA patients with portal hypertension (PH) (9.35 ± 2.3 ng/ml), than patients without PH (5.78 ± 1.9 ng/ml), and controls $p < 0.001$. There was a statistically significant positive correlation between serum level of GPC-3 and liver stiffness by fibroScan, ALT, AST, Total bilirubin, direct bilirubin, while there was a statistically significant negative correlation between serum level of GPC-3 and platelets, total protein, and serum Albumin.

CONCLUSION: In biliary atresia, serum GPC-3 is a valuable non-invasive marker for detecting deterioration of hepatic function and the degree of liver fibrosis.

Key words: Glypican 3; Liver stiffness; FibroScan; Biliary atresia

Behairy O.G, Abdelrahma A.M.N, Abdelshafy S.Z, Mahrous A.A.. Clinical Value of Serum Glypican-3 in Children With Biliary Atresia. *Journal of Gastroenterology and Hepatology Research* 2021; **10(6)**: 3619-3624 Available from: URL: http://www.ghrnet.org/index.php/joghr/article/view/3242

INTRODUCTION

Biliary atresia (BA) is an obstructive cholangiopathy that affects both the intrahepatic and extra-hepatic bile ducts in the first 3 months of life. Hepatomegaly, persistent jaundice and clay-colored stools are the main presentations. If BA left untreated, it is lethal with a reported survival rate of fewer than 10% at three years of age[1-2]. BA is thought to be a complex disease with endogenous and exogenous variables such as genetics, viral infections, toxins, and immunologic dysregulation being considered causative contributors[1].

BA can be classified into four phenotypes: isolated BA, BA associated with laterality anomalies (asplenia, polysplenia, abdominal situs inversus and intestinal malrotation), BA associated with other significant congenital anomalies, and BA associated with a bile duct

cyst (cystic BA)[3]. Due to a poorly defined disease pathophysiology, the main surgical treatment for BA is Kasai portoenterostomy (KPE); yet, most BA patients still require liver transplantation owing to progressive liver fibrosis toward cirrhosis[4].

This effect has motivated researchers to look for the molecular mechanisms driving this progression following KPE, as well as the development of, biochemical markers for recognizing the early stages of BA, which could assist patients to have a better prognosis and avoid the need for liver transplants[5].

Glypican-3 (GPC-3) is a member of the heparan sulfate proteoglycan family that forms glycosyl-phosphatidylinositol interaction with the plasma membranes external surface. Also, GPC-3 has been found to be overexpressed in several malignant tissues, most notably hepatocellular carcinoma (HCC)[6].

Due to its overexpression in HCC GPC-3 promote the growth of HCC by activation the Wnt signaling pathway. GPC-3 has emerged as a sensitive and specific marker for the diagnosis of HCC. This prompted us to investigate the idea that GPC-3 could be a great molecular predictor of biliary duct dysregulation[7]. Therefore, we conducted this study aiming to assess serum glypican-3 (GPC-3) levels in children with biliary atresia as well as the correlation of glypican-3 serum levels with clinical parameters.

METHODS

Study design and population

A total 100 patients were enrolled in this cross-sectional comparative study, which was separated into two groups: there were 50 children in the BA group, and their diagnosis was based on laparoscopy with surgical cholangiography (absence of dye excretion on intraoperative cholangiogram after ruling out other causes of obstructive jaundice). During the procedure, the gall bladders or the remnants of gall bladders were identified, but no extra-hepatic biliary duct was discovered and liver biopsy exhibited typical histopathological features (eg, bile plugs, duct proliferation, giant cell transformation, periportal fibrosis, and canalicular and cellular bile stasis)[8]. All patients underwent Kasai hepato portoenterostomy. None of them had undergone liver transplantation. Patients were recruited from the pediatric hepatology clinic of Benha University hospitals and National liver Institute, Menoufia University, between January 2020 and May 2021, along with 50 age/sex-matched healthy children as the control group. Control subjects were children who went to an outpatient clinic for a regular checkup for athletic training or school routine examination.

The exclusion criteria included any patient with other chronic liver diseases as: viral causes as (Hepatitis B&C), Cytomegalovirus (CMV), Epstein Barr virus (EBV). Toxic and drugs as: (Alcoholic liver disease, Methotrexate, Amiodarone &Nitrofurantoin). Metabolic as: (Non-alcoholic fatty liver disease, Haemochromatosis, Wilson's disease). Autoimmune response causes as: (Primary biliary cholangitis, Primary sclerosing cholangitis, Autoimmune Hepatitis). The study was approved by the Ethical Committee of Faculty of Medicine, Benha University which followed ''The World Medical Association's Code of Ethics'' (Declaration of Helsinki). Parents/guardians gave their consent after being fully informed about all study protocols before enrolment.

All children were subjected to the following

1. Complete medical history and physical examination (general and abdominal examinations) with a focus on clinical manifestations (jaundice, clay-colored stool, abdominal pain and distension, melena, and other bleeding such as hematuria, epistaxis, or bleeding gums).

2. Abdominal ultrasonography is used to determine the size and texture of the liver as well as the existence of ascites, and spleen's size.

3. For the biliary atresia group transient elastography (FibroScan, Echosens, Paris, France) was used to quantify the stiffness of the liver between 25 and 65 mm from the skin surface, which is about similar to the volume of a cylinder 1 cm in diameter and 4 cm in length. The measurements were taken with patients sleeping in a dorsal decubitus position with maximum abduction of the right arm and fibroScan transducer probe placed on the intercostal space near the right lobe of the liver. A section of the liver that was at least 6 cm thick and free of significant vascular structures was chosen as the measuring target. The measurements were carried out until a total of ten validated results were achieved with a success rate of at least 80%. The elastic modulus of the liver was calculated using the median value of the 10 validated scores and expressed in kilopascals (kPa).

4. Laboratory investigations. Sampling: Six milliliters of venous blood were taken from each participant via peripheral venipuncture under aseptic conditions and divided as follows: 1mL of blood in 150mL ethylene diamine tetra acetic acid to perform complete blood count by Sysmex-XP300. The remaining blood was centrifuged for 10 minutes and the serum was used for chemical testing which included: serum aminotransferases (aspartate aminotransferase [AST]-alanine aminotransferase [ALT]), gamma-glutamyl transferase, alkaline phosphatase, bilirubin, albumin, and total protein, all of which were performed on Biosystem A15 autoanalyzer (Barcelona, Spain).

The remaining serum was stored at -20^{0}C serum GPC-3 level determination using an ELISA sandwich technique purchased from the Sunredbio Co (Shanghai, China) with (Cat No: E1431Hu).

Statistical analysis

The SPSS (statistical program for social science version 24) was used to analyze the data as follows: the mean, SD, and range of quantitative data are used to describe them. The numbers and percentages are used to describe qualitative data. When appropriate the chi-square, Student's unpaired *t*-test, and Mann Whitney test were used to compare demographic and clinical parameters between groups. Pearson's correlation coefficient (*r*) was used to determine the correlation between numerical data. Glypican 3 cutoff values with the best sensitivity and specificity in predicting fibrosis in biliary atresia were determined using a ROC curve. All P value less than 0.05 are considered statistically significant.

RESULTS

The present study included 100 subjects who were divided into 2 groups; Biliary atresia group: included 50 children, they were (23girls/27 boys) with Mean ± SD age (26.4 ± 15.3months) ranged from 3-72 months and control group consists of 50 children, they were 25 males and 25 females, their mean age was 20.7 ± 14.9 ranged from 3-72 months. There was no significant difference in age or gender between BA and control groups (P-value = 0.231, 0.729 respectively).

Regarding clinical presentation in BA patients, according to their jaundice status (total bilirubin): they were 33 (66%) children were non-jaundiced (TB < 2 mg/dl) and 17(33%) children had persistent jaundice (TB ≥ 2 mg/dl), portal hypertension (PH) was assessed by the presence of ascites and/or esophageal varices observed by endoscopy, as they were 21patients (42%) had ascites, and 20 patients

(40%) had esophageal varices. Mean ± SD liver stiffness values by transient elastography (fibroScan) in BA subjects was 15.4 ± 6.9, as they were 6.4 ± 0.4 kPa for F1, 8.6 ± 1.3 kPa for F2, 13.9 ± 1.5 kPa for F3, and 22.2 ± 2.7 kPa for F4.

The baseline characteristics of BA patients and control subjects are presented in (Table 1). As there was a statistically significant increase in liver function tests in the biliary atresia group than the control group.

Serum Glypican 3 assessment

Serum GPC-3 was statistically higher in the biliary atresia group (7.26 ± 3.48 ng/ml) than the control group (1.7 ± 0.52 ng/ml), $p < 0.001$ (Table 1, Figure 1). Also, it was higher in BA with jaundice than BA without jaundice and controls. Moreover, GPC-3 was higher in BA patients with portal hypertension (PH), than patients without PH, and controls (Table 2).

Serum GPC-3 was statistically higher in patients with ascites splenomegaly and in patients with esophageal varices, while there was no statistically significant difference regarding sex (Table 3).

There was a statistical significant positive correlation between serum level of GPC-3 spleen size (0.644, $p < 0.001$), degree of fibrosis by fibroScan (0.766, $p < 0.001$), ALT (0.588, $p < 0.001$) AST (0.443, $p < 0.001$) GGT (0.521, $p < 0.001$) , Total bilirubin (0.769, $p < 0.001$) direct bilirubin (0.768, $p < 0.001$), Alkaline phosphatase (0.555, $p < 0.001$), PT (0.548, $p < 0.001$), and INR (0.527, $p < 0.001$). While there was a statistical significant negative correlation between serum level of GPC-3 and hemoglobin (-0.305, $p < 0.001$), platelets (-0.524, $p < 0.001$), total protein (-0.384, $p < 0.001$) and serum Albumin (-0.605, $p < 0.001$).

Table 1 biochemical characteristics of the studied groups.

		Biliary atresia group		Control group		Test	P value
		N=50	%	N=50	%		
ALT (IU/L)	Median	204		30		U=7.4	<0.001
	IQR	187-214		27-33			
AST (IU/L)	Median	152		21		U=7.5	<0.001
	IQR	130-168		20-24			
GGT (IU/L)	Median	1026		26		U=7.3	<0.001
	IQR	965-1250		20-30			
Alkaline phosphatase (IU/L)	Median	578		205		U=7.1	<0.001
	IQR	547-632		170-210			
Protein (g/L)	Mean ±SD	6.1±0.8		6.4±0.5		t=2.1	0.04
	Range	4.5-8		5.7-7.5			
Albumin (g/L)	Mean ±SD	2.6±0.3		4.4±0.4		t=8.6	<0.00
	Range	2.1-4.5		3.7-5.2			
Bilirubin total (mg/dL)	Median	7.5		1		U=6.7	<0.00
	IQR	6.3-9.7		1-1.1			
Bilirubin direct (mg/dL)	Median	4.1		0.06		U=6.8	<0.00
	IQR	3.4-5		0.05-0.20			
Hb (gm/dl)	Mean ±SD	10.9±1.9		12.2±1.2		t=3.4	<0.001
	Range	7.5-14.6		9.5-15			
WBCs (l)	Mean ±SD	11.8±3		10.5±2		t=0.55	0.57
	Range	7.3-23		8-12			
PLT (103/l)	Mean ±SD	202±36		283±46		t=8.8	<0.001
	Range	139-291		208-356			
PT (Seconds)	Mean ±SD	13.7±1.5		12.6±0.5		t=3.6	<0.001
	Range	12-17.8		12-13.4			
INR	Mean ±SD	1.23±0.22		1.09±0.07		t=3.5	<0.001
	Range	1-1.89		1-1.18			
Glypican 3 (ng/ml)	Mean ±SD	7.26±3.48		1.7±0.52		t=8.7	<0.001
	Range	1.85-14.3		0.87-2.78			

t: Student t-test, U: Mann-Whitney test, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: Gamma Glutamyl Transferase. Hb: Hemoglobin, WBCs: white blood cells, PLT: platelets, PT: prothrombin time, INR: international normalized ratio

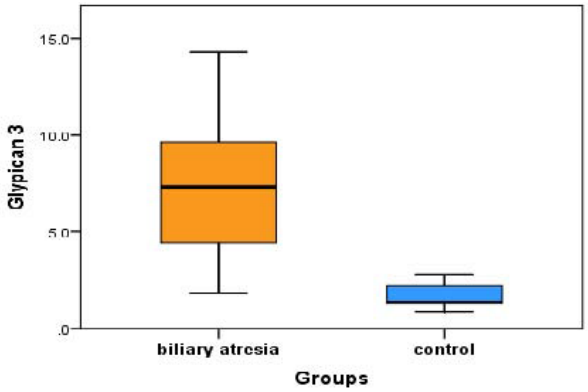

Figure 1 Comparison between biliary atresia group and control group regarding serum Glypican 3.

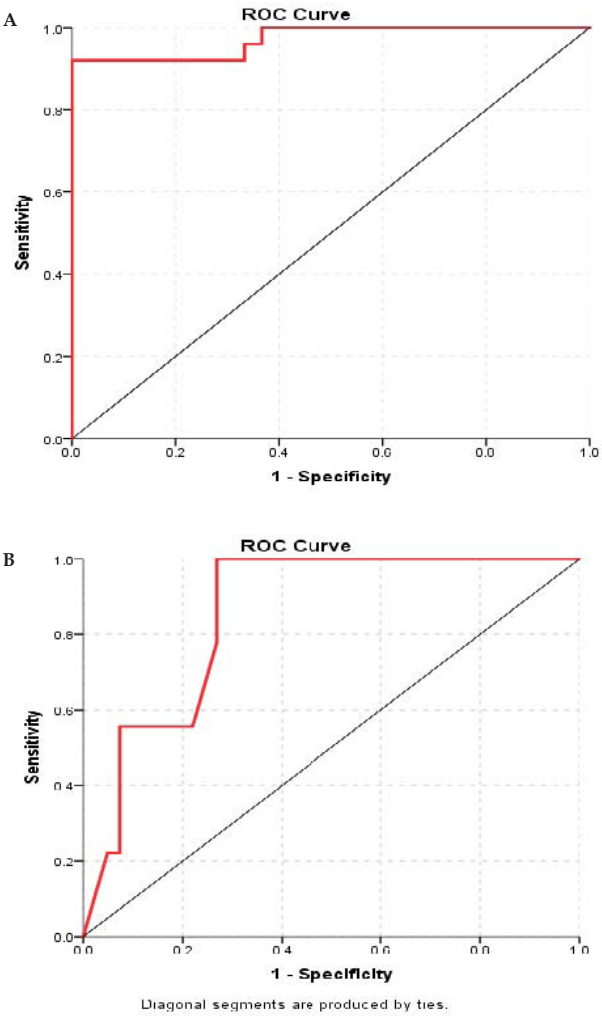

Figure 2 A: ROC curve of performance of serum levels of Glypican 3 for detection of fibrosis (>F0) in cases with biliary atresia. B: ROC curve of performance of serum levels of Glypican 3 to detect fibrosis (≥ F3) in cases with biliary atresia.

Table 2 Comparison between serum glypican 3 in BA with jaundice, portal hypertension and without jaundice, portal hypertension and controls

		Glypican 3 (ng/ml)		Test	P value
		Mean ±SD	Range		
Biliary atresia group	With jaundice (N=33)	9.1±2.66	1.85-14.3	F=137.8	<0.001 a,b,c
	Without jaundice (N=17)	3.6±1.2	1.85-5.33		
Control (N=50)		1.7±0.52	0.87-2.78		
Biliary atresia group	With portal hypertension (N=20)	9.35±2.3	5.62-14.3	F=61.4	<0.001 a,b,c
	Without portal hypertension (N=30)	5.78±1.9	1.85-14.1		
Control (N=50)		1.7±0.52	0.87-2.78		

F: F-value of one-way anova, a; significance difference between group with jaundice, portal hypertension& without jaundice, portal hypertension, b; significant difference between with jaundice, portal hypertension & control, c: significance difference between group with without jaundice, portal hypertension & control.

Table 3 Comparison between serum Level of Glypican 3 and some clinical parameters.

		Glypican 3 (ng/ml)		Test	P value
		Mean ±SD	Range		
Sex	Male	4.7±3.67	1.24-14.3	t=0.922	0.341
	Female	5.6±4.09	0.87-13.02		
Ascites	Yes	9.34±2.16	5.62-14.3	t=6.9	<0.001
	No	4.39±2.89	1.85-14.3		
Splenomegaly	Enlarged	9.36±2.40	5.09-14.30	t=8.5	<0.001
	Average	3.84±1.86	1.85-9.63		
Endoscope	Esophageal varices	9.35±2.33	5.62-14.3	t=6.7	<0.001
	Free	5.87±3.45	1.85-14.30		

Comparison between the jaundiced group and the non-jaundiced group of biliary atresia patients

Children with BA with jaundice exhibited significantly higher age, direct bilirubin, serum GPC-3 values, and liver stiffness measurement (fibroScan) than children with BA without jaundice (Table 4).

Diagnostic performance and predictive value of serum Glypican 3

The diagnostic performances of GPC-3 to detect the presence of fibrosis (> F0) in children with BA indicated that serum GPC-3 at a cut-off point ≥ 2.6 ng/ml had a 93% sensitivity, 96.7% specificity with a fair area under the ROC curve (AUC) of 0.972 (95% confidence interval: 0.942-1), $p < 0.001$. While at cut-off point ≥ 8.7 ng/ml with 88.9% sensitivity, 73.2% specificity, with a fair area under the ROC curve (AUC) of 0.953 (95% confidence interval: 0.75-0.953), $p < 0.001$ serum GPC-3 could detect severe fibrosis (≥ F3) (Figure 2).

Table 4 Comparison of Biliary atresia patients with and without jaundice

		Biliary atresia group				Test	P value
		With jaundice		Without jaundice			
		N=33	%	N=17	%		
Sex	Male	17	51.50%	10	58.80%	X^2= 3.7	0.32
	Female	16	48.50%	7	41.20%		
Age (months)	Mean ± SD	37.7±19.5		5.9±2.1		t=6.6	<0.001
Hemoglobin (gm/dL)	Mean ± SD	10.8±1.7		10.9±2.1		t=0.27	0.812
WBCs (L)	Mean ± SD	12.6±3.4		13.1±2.1		t=0.42	0.62
Platelets (10^3/L)	Mean ± SD	199±32		207±42		t=0.79	0.46
ALT(IU/L)	Mean ± SD	242.3±132.2		201.4±39.1		t=1.19	0.12
AST(IU/L)	Mean ± SD	167.7±80.9		164.3±40.7		t=0.13	0.87
GGT(IU/L)	Mean ± SD	1139±641		1041±251		t=0.31	0.7
Bilirubin-Direct (mg/dL)	Mean ± SD	5.7±2.3		0.68±0.32		t=8.9	<0.001
Alkaline phosphatase (IU/L)	Mean ± SD	583±207		601±80.5		t=0.45	0.56
Albumin (g/L)	Mean ± SD	3.5±0.32		3.7±0.45		t=1.99	0.09
Glypican 3 (ng/mL)	Mean ± SD	9.1±2.66		3.6±1.2		t=8.1	<0.001
Fibroscan (kPa)	Mean ± SD	14.5±3.3		11.3±3.7		t=2.7	<0.003

X2: Chi-square test, t: Student t-test, dALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: Gamma Glutamyl Transferase. Hb: Hemoglobin, WBCs: white blood cells, PLT: platelets.

DISCUSSION

All preoperative testing is inaccurate, and the current diagnostic approaches for BA diagnosis rely on invasive procedures such as surgical exploration and operative cholangiogram. Even though the Kasai operation can drain bile, it has a short therapeutic window, and many patients continue to have difficulties such as chronic cholestasis, portal hypertension, liver fibrosis, and cirrhosis. Continuous total bilirubin readings are required for the monitoring of persistent jaundice, but liver fibrosis is generally diagnosed with liver biopsy. As a result, noninvasive techniques that encourage early detection and recognition of complications are beneficial. Serum biomarkers have been widely employed as a screening or prognostic tool for different disorders, such as degenerative and malignant diseases[9-10].

In the present study, we investigated circulating GPC-3 levels in BA patients compared to healthy controls as well as associations between circulating GPC-3levels and clinical parameters of hepatic dysfunction in BA patients and we found that GPC-3 levels were statistically higher in the biliary atresia group than the control group. In addition, BA with jaundice and portal hypertension had a greater rate than BA without jaundice and portal hypertension. This was in agreement with the finding of Sirisombonnlarp et al[11] who found that circulating GPC-3 levels were significantly higher in BA patients than in healthy controls (8.7 ± 0.1 vs. 6.5 ± 0.3 ng/ml, $p < 0.001$), and also significantly higher in jaundiced BA patients than in non-jaundiced BA patients ($p < 0.001$).

Up-regulation of glypican-3 mRNA expression was also detected in biliary atresia livers according to Udomsinprasert et al[5] and glypican-3 immunostaining scores were significantly elevated in biliary atresia livers. Patients with biliary atresia who had poor outcomes exhibited significantly higher glypican-3 expression than those who had favorable outcomes, and they concluded that increased expression of glypican-3 was linked to poor survival[5].

GPC-3 is involved in a wide range of physiological processes, including cell proliferation, differentiation, and apoptosis[12]. GPC-3 is an unexpected regulator of cell proliferation and morphogenesis particularly in hepatocytes [13]. GPC-3 has been found to accelerate the growth of HCC by activating the Wnt/b-catenin signaling pathway through its effect on hepatocyte morphogenesis[14]. The Wnt/ b-catenin signaling pathway has been discovered to be one of the major

mechanisms regulating biliary tracts morphogenesis in BA[15], potentially establishing a potential physiologic action of GPC-3 interacting with the Wnt/b-catenin signaling in the development of bile ducts involving BA pathology. This finding provides that GPC-3 plays a regulatory function in the development of liver fibrosis in BA which is mediated by Wnt/b-catenin signaling[5].

Capurro and colleagues[14], studied the effect of GPC3 on HCC cell lines and found that GPC3 promotes HCC growth by activation of canonical Wnt signaling, making GPC-3-mediated Wnt signaling an appealing treatment. Notably, because Notum, a lipase that cleaves glycosyl-phosphatidylinositol (GPI) anchors can release GPC3 into the circulation from the cell surface; it may have diagnostic utility as a circulating marker for human illnesses[16]. Chen et al[17] also found that circulating GPC3 levels in patients with HCC were significantly higher than in both patients with other liver diseases and healthy controls.

All of these findings are consistent with our study which found that circulating GPC3 levels in BA patients are statistically higher than in healthy controls and those children with advanced BA, including jaundice and portal hypertension, have significantly higher circulating GPC3 levels than those in earlier stages. Also, in the present study GPC-3 levels were positively correlated with liver function tests (AST, ALT, GGT, bilirubin total and direct, alkaline phosphatase, PT, INR,) spleen size and degree of fibrosis by fibroScan in BA patients. While there were statistically significant negative correlations between serum level of GPC-3 and hemoglobin, platelets, total protein, and serum Albumin.

These findings are consistent with those of[5,11] who reported a positive correlation between GPC-3 levels and liver stiffness and liver enzymes, concluding that elevated serum GPC3 levels were associated with hepatic dysfunction and the severity of BA. As a result, serum GPC3 and liver stiffness could be used as indicators for deterioration of hepatic function and prognosis in post-Kasai BA.

Shirakawa et al[18] found that hepatic GPC-3 protein expression was negatively associated with outcome parameters such as age and serum albumin, but that expression levels were positively correlated with ALP values, AST, and the degree of liver fibrosis. Further research revealed that BA patients with high GPC-3 protein expression had lower survival than those with low GPC-3 protein expression. This finding is supported by previous research that linked high GPC-3 protein expression to a lower chance of survival in patients with HCC. Given our findings, it's tempting to speculate that elevated hepatic expression of GPC-3 protein in BA patients and those in advanced stages reflects the body's defensive response to hepatic dysfunction or hepatocellular injury, resulting in its overexpression in BA patients. Furthermore, the destruction of biliary ducts may result in overexpression of GPC-3 which leads to an increase in its synthesis via GPC-3 interacting with Wnt signaling[19].

An increase in circulating levels of GPC3 in BA patients, particularly those with a poor outcome, could be due to an imbalance between GPC3 synthesis and its clearance, In this setting, an increase in circulating GPC3 levels could be part of the body defense reaction to hepatocellular damage and subsequent hepatic fibrogenesis. Furthermore, the destruction of biliary ducts a feature unique to BA compared to controls may result in a reduction in GPC3 clearance which would raise GPC-3 levels in BA serum[11]. GPC-3 may have a role in the process of hepatocellular injury and hepatic fibrogenesis in postoperative BA patients, and it may also be implicated in the degree of bile duct obliteration, according to these data. As a result, it's fair to believe that circulating GPC3 could be developed as a non-invasive biomarker detecting BA disease progression[11].

CONCLUSION

Serum GPC-3 could be a promising non-invasive biomarker for the detection of deterioration of hepatic function and the degree of liver fibrosis in biliary atresia. Larger-scale studies may be required to corroborate these findings.

REFERENCES

1. Vij M, Rela M. Biliary atresia: pathology, etiology and pathogenesis. *Future Sci OA*. 2020 Mar 17; **6(5)**: FSO466. [PMID: 32518681]; [PMCID: PMC7273417]; [DOI: 10.2144/fsoa-2019-0153]
2. LeeVan E, Matsuoka L, Cao S, Groshen S, Alexopoulos S. Biliary-Enteric Drainage vs Primary Liver Transplant as Initial Treatment for Children With Biliary Atresia. *JAMA Surg*. 2019 Jan 01; **154(1)**: 26-32. [PMID: 30208381]; [PMCID: PMC6439864]; [DOI: 10.1001/jamasurg.2018.3180]
3. Sokol RJ, Shepherd RW, Superina R, Bezerra JA, Robuck P, Hoofnagle JH. Screening and outcomes in biliary atresia: summary of a National Institutes of Health workshop. *Hepatology*. 2007; **46**: 566-581. [PMID: 17661405]; [PMCID: PMC3888317]; [DOI: 10.1002/hep.21790]
4. Sundaram SS, Mack CL, Feldman AG, Sokol RJ. Biliary atresia: Indications and timing of liver transplantation and optimization of pretransplant care. *Liver Transpl.* 2017; **23(1)**: 96-109. [PMID: 27650268]; [PMCID: PMC5177506]; [DOI: 10.1002/lt.24640]
5. Udomsinprasert W, Angkathunyakul N, Klaikeaw N, Vejchapipat P, Poovorawan Y, Honsawek S. Hepatic glypican-3 and alpha-smooth muscle actin overexpressions reflect severity of liver fibrosis and predict outcome after successful portoenterostomy in biliary atresia. *Surgery.* 2020; **167(3)**: 560-8. [PMID: 31761395]; [DOI: 10.1016/j.surg.2019.10.013]
6. Guo M, Zhang H, Zheng J, Liu Y. Glypican-3: a new target for diagnosis and treatment of hepatocellular carcinoma. *J Cancer.* 2020; **11(8)**: 2008. [PMID: 32127929]; [PMCID: PMC7052944]; [DOI: 10.7150/jca.39972]
7. Gao W, Ho M. The role of glypican-3 in regulating Wnt in hepatocellular carcinomas. *Cancer Rep*. 2011; **1(1)**: 14. [PMID: 22563565]; [PMCID: PMC3343874]
8. Esmaili J, Izadyar S, Karegar I, et al. Biliary atresia in infants with prolonged cholestatic jaundice: diagnostic accuracy of hepatobiliary scintigraphy. *Abdom Imaging* 2007; **32**: 243-7. [PMID: 16967250]; [DOI: 10.1007/s00261-006-9049-4]
9. Wang, J. *et al.* Liver immune profiling reveals pathogenesis and therapeutics for biliary atresia. *Cell* 2020; **183**, 1867-1883.e1826. [PMID: 33248023]; [DOI: 10.1016/j.cell.2020.10.048]
10. Elsharkawi, F., Elsabah, M., Shabayek, M. & Khaled, H. Urine and serum exosomes as novel biomarkers in detection of bladder cancer. *Asian Pac. J. Cancer Prev* 2019; **20**: 2219-2224. [PMID: 31350988]; [PMCID: PMC6745236]; [DOI: 10.31557/APJCP.2019.20.7.2219]
11. Sirisomboonlarp K, Udomsinprasert W, McConachie E, Woraruthai T, Poovorawan Y, Honsawek S. Increased serum glypican-3 is associated with liver stiffness and hepatic dysfunction in children with biliary atresia. *Clin Exp Hepatol.* 2019; **5(1)**: 48-54. [PMID: 30915406]; [PMCID: PMC6431085]; [DOI: 10.5114/ceh.2019.83156]
12. Liu B, Paranjpe S, Bowen WC, et al. Investigation of the role of glypican 3 in liver regeneration and hepatocyte proliferation. *Am J Pathol*. 2009; **175**: 717-724. [PMID: 19574424]; [PMCID: PMC2716967]; [DOI: 10.2353/ajpath.2009.081129]
13. Grozdanov PN, Yovchev MI, Dabeva MD. The oncofetal protein glypican-3 is a novel marker of hepatic progenitor/oval cells. *Lab Invest*. 2006; **86**: 1272-1284. [PMID: 17117158]; [DOI: 10.1038/labinvest.3700479]
14. Capurro MI, Xiang YY, Lobe C, Filmus J. Glypican-3 promotes

the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res*. 2005; **65**: 6245-6254. [PMID: 16024626]; [DOI: 10.1158/0008-5472.CAN-04-4244]
15. Decaens T, Godard C, de Reyni_es A, et al. Stabilization of beta-catenin affects mouse embryonic liver growth and hepatoblast fate. *Hepatology*. 2008; **47**: 247-258. [PMID: 18038450]; [DOI: 10.1002/hep.21952]
16. Traister A, Shi W, Filmus J. Mammalian Notum induces the release of glypicans and other GPI anchored proteins from the cell surface. *Biochem J* 2008; **410**: 503-511. [PMID: 17967162]; [DOI: 10.1042/BJ20070511]
17. Chen M, Li G, Yan J, et al. Reevaluation of glypican-3 as a serological marker for hepatocellular carcinoma. *Clin Chim Acta* 2013; **423**: 105-111. [PMID: 23643963]; [DOI: 10.1016/j.cca.2013.04.026]
18. Shirakawa H, Suzuki H, Shimomura M, et al. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. *Cancer Sci*. 2009; **100**: 1403-1407. [PMID: 19496787]; [DOI: 10.1111/j.1349-7006.2009.01206.x]
19. Wands JR, Kim M. WNT/b-catenin signaling and hepatocellular carcinoma. *Hepatology*. 2014; **60**: 452-454. [PMID: 24644061]; [DOI: 10.1002/hep.27081]