

Short Communication

Galectin-3-Binding Protein as a biomarker of choledochal cyst in children: A pilot study

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ABSTRACT

A choledochal cyst (CDC) is a rare congenital, single, or multiple cystic dilatations of the biliary tract encountered most commonly in infants and children. A sensitive and specific serological marker, if available, may add to its early recognition thereby avoiding the risk of complications frequently seen in these patients. We analyzed serum galectin-3-binding protein (Gal-3BP) levels in 21 children with CDC and 14 age-matched controls by the ELISA method. Serum Gal-3-BP levels were significantly raised in the patient group (36.6 ± 25.2 ng/ml versus 7.0 ± 4.3 ng/ml in controls; $p < 0.0001$) and 95.2% of patients had Gal-3BP levels higher than the mean value in controls. Almost 67% of patients showed values ≥ 3 times of mean Gal-3BP level in controls ($p < 0.0001$). Equal to 5 times or greater levels were seen in 43% of the patients ($p = 0.0051$). The study, therefore, suggests further evaluation of Gal-3BP as a potential biomarker for CDC and other biliary disorders.

Keywords: Galectin-3-binding protein, Choledochal cyst, Serological biomarker.

A choledochal cyst (CDC) is a rare disease presenting as cystic dilatation of the bile duct. About 80% of CDC cases are diagnosed in infancy and adolescence.(1) The disease can present as chronic or intermittent abdominal pain, biliary cirrhosis, or can be asymptomatic. Occasionally complications may occur in the form of recurrent cholestasis, cholangitis, cholelithiasis, common bile duct perforation, pancreatitis, right upper quadrant mass, or its malignant transformation.(2) No drug or conservative treatment is available for CDC hence, surgical excision of the cyst and Roux-en-Y hepatic enterostomy or hepatic duodenostomy is the preferred practice. Besides surgical removal, the treatment also aims to avoid CDC-associated hepatic, pancreatic, or biliary complications.

Since there is always a risk of complications, its early recognition is of paramount importance and largely relies on ultrasonography (USG) than the other high ends

radiological diagnostic methods, which are not feasible for screening of the high-risk population.(3) Availability of an easier and more reliable laboratory test, as an ancillary diagnostic tool, may play a crucial role in the disease management and prognosis of CDC.

Galectin-3-binding protein (Gal-3BP), also called basement membrane autoantigen p105 or Mac-2-binding protein is a ubiquitous and multifunctional glycoprotein. Gal-3BP is not only present in serum but also found in tears, saliva, breast milk, semen, etc.(4) Raised serum Gal-3BP levels are associated with hepatocellular carcinomas, pancreatic adenocarcinoma, biliary tract carcinoma, and many other tumor entities.(5)

We aimed to evaluate the efficacy of Gal-3BP as a potential biomarker of CDC for which 21 patients of CDC with age ≤ 12 years and 14 healthy children of comparable age were recruited. A diagnosis of CDC was confirmed by clinical history, biochemical laboratory findings such as

liver function tests, and radiological investigations like USG, and magnetic resonance cholangiopancreatography (MRCP). Patients with other causes of cholestasis or neonatal hepatitis were excluded. Informed consent was obtained from the parents/guardians of all study subjects for their participation and the study was approved by the Institute's Human Ethics committee (No: INT/IEC/SPL-1402-A).

About 2 ml of peripheral blood was withdrawn and collected in a red-top vacutainer from controls and CDC patients just before surgery. The serum was separated from the whole blood by centrifuging at 2000g for 10 minutes at 4 °C, aliquoted in a quantity of 500 µl, and stored at -80 °C.

Gal-3BP was detected in serum samples using commercially available ELISA kits (Elabscience®, Houston, Texas, USA, Catalogue number E-EL-H1456). The test procedure was followed as per the manufacturer's instructions. Briefly, 100 µl of diluted standards, blanks, and serum samples were added in appropriate wells and incubated for about 90 min at 37°C. The liquid was then decanted and to each well, 100 µl of biotinylated detection antibody was added followed by another 60 minutes incubation at 37°C. The wells were washed with buffer followed by the addition of 100 µl enzyme conjugate and further incubated for 30 minutes. After washing the unbound enzyme conjugates and adding substrate solution, an additional 15 min incubation at 37°C was carried out. Finally, a stop solution was added and the optical density was measured at 450 nm in an ELISA reader. A cut-off value and antibody index were calculated as suggested by the manufacturer and the data was interpreted. The serum concentration of Gal-3BP was calculated by plotting a four-parameter logistic curve on the log-log axis of known standards as per the kit manual, and a comparative analysis was carried out.

Statistical analysis was done with the help of GraphPad Prism statistical software, 8.0.2. The demographic or clinical parameters between the study groups were compared using Chi-square, Fisher's exact, Student's unpaired t-test, or Mann-Whitney U test. The data has been shown as a mean SEM or as a percentage. A p-value <0.05 was considered statistically significant.

Among the 21 patients, 12 were males and nine females with a mean age of 6.1 years (range 2-12 years) and a male to female ratio of 1.44:1. The mean age among the five male and nine female controls was 7.2 years (range 2.6-12 years) and the male to female ratio was 1.91:1. All patients had raised levels of serum bilirubin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase.

Serum Gal-3BP was significantly elevated in patients with CDC (36.60 ±25.2 ng/ml) than the controls (7.0 ±4.3 ng/ml) with a p-value <0.0001 (Figure 1A). The Gal-3BP value in patients ranged from 2.2 to 87.8 ng/ml,

while in controls it was from 0.6 to 13.4 ng/ml. When calculated against the mean ±3SD Gal-3BP levels of controls, 15(71%) patients had higher levels, while in the control group, the serum Gal-3BP was lower than the mean ±3SD (Figure 1B). Also, 20(95.2%) patients showed higher Gal-3BP levels compared to mean Gal-3BP levels in controls (p = 0.0032). Eighteen patients (85.7%) had ≥2 times the mean control levels, while 14(66.7%) of them had levels of ≥3 times the mean Gal-3BP (p <0.0001). Interestingly, ≥5 times values were observed in 9(42.8%) patients (p = 0.0051; Table 1).

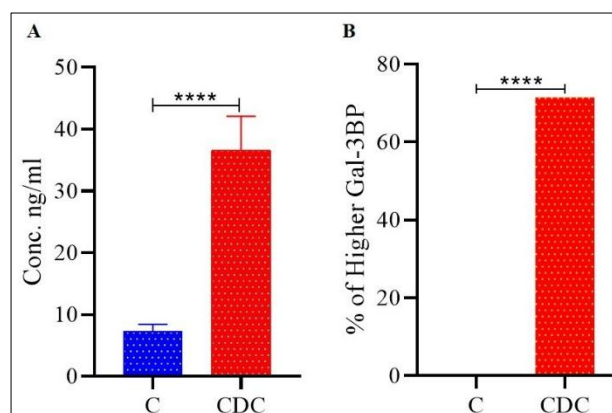


Figure 1. Serum Gal-3BP levels in the study groups. A) Comparison of mean Gal-3BP in patients versus controls. B) Percentage of patients and controls with raised Gal-3BP level when compared against mean ±3SD value of controls. (MannWhitney U test has been used for the comparative analysis of Gal-3BP levels and Fisher's exact test for positivity analysis. pvalue was calculated at 0.05)

Gal-3BP has been reported to have a pathogenic role in diseases like asthma, rheumatoid arthritis, non-alcoholic fatty liver disease (NAFLD), diabetes, atherosclerosis, venous thrombosis, etc. Its receptor/ligand Gal-3 on the other hand has been shown to have diagnostic or prognostic value in various disorders such as heart and kidney diseases, viral infections, autoimmune disorders, neurodegenerative diseases, and cancers.(6–10)

Although Gal-3BP has recently been proposed as an early biomarker of obesity, metabolic syndrome, and glioma, its association with CDC so far has not been reported. The present study surprisingly showed high Gal-3BP levels in all our test subjects.(11,12)

USG is a noninvasive, cheap, and very effective tool in detecting cystic structures hence is the most preferred imaging choice for CDC. Its results however are observer-dependent and sometimes it may pose difficulty in commenting on the biliary origin of the cyst or a concomitant anomalous pancreaticobiliary junction. It becomes difficult for USG to differentiate CDC from biliary atresia, a neonatal disorder that can also present as a cyst and requires urgent surgical intervention. Also, in adult patients' differentiation of CDC from other benign or malignant conditions leading to bile duct dilatation may not be easy by USG alone.(13) Multimodality imaging modali-

ties like computed tomography (CT) scan, magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP) also help in planning the surgical management but have their limitations e.g., exposure to radiation, contrast media, the need for general anesthesia, and risk of complications, etc. Hence, except USG none of these techniques are suitable for routine screening in these patients.

Table 1: Comparative analysis of Gal 3BP levels in the study groups

Study Subjects	Gal-3BP levels					
	≥ Mean	≥ 2x Mean	≥ 3x Mean	≥ 4x Mean	≥ 5x Mean	≥ Mean +3SD
CDC	95.2% (20/21)	85.7% (18/21)	66.7% (14/21)	52.4% (11/21)	42.8% (9/21)	71.4% (15/21)
Controls	50.0% (7/14)	7.1% (1/14)	0%	0%	0%	0%

CDC if undiagnosed may end up with associated complications hence its treatment is focused on their prevention. An early diagnosis, therefore, plays a crucial role and may alter the course of management of the CDC. A serological marker, if available, may be used as an ancillary tool, particularly in smaller, resource-limited, or remotely situated healthcare centers. An advantage is that being a simple enzyme immunoassay, the patient does not need not to visit as a blood sample can be sent to the testing laboratory. Also, the testing cost of a serological assay is comparable to USG and cheaper than the other imaging methods sometimes needed for diagnosis.(3) We found that Gal-3BP was highly sensitive in detecting CDC as 95% of the patient's levels were higher than the controls. However, for us, it is not possible to comment on the specificity of this molecule unless a larger study is conducted including other differential conditions leading to cholestasis.

Recently, Ming et al., in a proteomic analysis in three patients of CDC found fructose-bisphosphate aldolase B (ALDOB) among the upregulated protein in patients of CDC.(3) Gal-3BP can be used in conjunction with

ALDOB as biomarkers to give a more accurate diagnosis of CDC, but the role of ALDOB is yet to be proven.

In agreement with our findings, raised Gal-3BP levels have also been reported in cirrhosis and hepatocellular carcinoma.(14) It also is associated with the severity of non-alcoholic steatohepatitis (NASH). Gal-3BP might as well be useful in the diagnosis of NASH and could predict fibrosis in chronic liver diseases and NAFLD.(15,16) Cheung et al., in another proteomic study, reported that serum Gal-3BP can be used as a biomarker of fibrosis progression in HCV patients.(17) Given the higher serum value of Gal-3BP at the time of surgical excision of the CDC, it might be possible that it is indicative of liver fibrosis and may correlate with disease severity. Based on the small sample size which is a major limitation in the present study, definitive conclusive opinions cannot be drawn-out. However, the significance of this study is that it provides little hope in the form of Gal-3BP for the patients of CDC and possibly other cholestatic disorders of similar age groups.

To conclude, in a very small pilot study, we found highly elevated serum GAL-3BP levels in ≤12 years old patients with CDC. The finding advocates further exploration of this molecule for its utility as a biomarker of the CDC. Future studies on a larger cohort, if carried out in addition, may shed some light on the usefulness of Gal-3BP in differentiating CDC from other cholestatic diseases and its significance in other hepatobiliary disorders.

Conflict of Interest: Nil

Source of Support: Nil

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Authors Contribution: Author(s) declared to fulfill authorship criteria as devised by ICMJE and approved the final version. Authorship declaration form, submitted by the author(s), is available with the editorial office.

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