

# Usefulness of clinical and laboratory parameters for differentiation of biliary atresia from idiopathic neonatal hepatitis: Experience in a tertiary care hospital of Bangladesh

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## Abstract

**Background:** The two most common and important causes of neonatal cholestasis (NC) are biliary atresia (BA) and Idiopathic neonatal hepatitis (INH). There is no single test that can definitely differentiate these two entities. **Objective:** To evaluate the diagnostic accuracy of clinical and laboratory parameters for diagnosis of biliary atresia. **Methods:** This cross-sectional study was conducted at the department of Pediatric Gastroenterology and Nutrition of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, from August 2013 through July 2015 among purposively sampled infants with neonatal cholestasis. **Results:** Total 86 neonatal cholestatic cases were studied. Term baby and good birth weight are significantly higher in BA cases. The presence of persistent pale colored stool is significantly more in patients with BA (p 0.000). GGT is the only liver enzyme that was found to be useful differentiating BA from INH at a cut-off value  $\geq 524$ U/L or 9.5 times higher than upper limit normal with sensitivity and specificity of 81.6% and 72.9% respectively. In the present study the diagnostic accuracy of persistent pale colored stool found to be highest (79.1 %).

**Conclusion:** The present study showed that persistent pale colored stool and serum level of GGT with a cut-off value  $\geq 524$  U/L or 9.5 times higher than upper limit normal can be considered as predictive markers for differentiation of Biliary atresia from Idiopathic neonatal hepatitis.

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**Key words:** Neonatal cholestasis, Biliary atresia, Idiopathic neonatal hepatitis, clinical features, Liver biopsy.

## Introduction

Neonatal cholestatic (NC) infants present with prolonged conjugated hyperbilirubinemia, passage of dark urine, pale stools and enlarged liver which are nonspecific features. The frequency of NC in the population is difficult to evaluate. Incidences of 1/2,500, 1/5,000, and 1/9,000 live births have been recorded in different literatures<sup>1-4</sup>. The two most common and important causes of neonatal cholestasis (NC) are biliary atresia (BA) and Idiopathic neonatal hepatitis (INH)<sup>5</sup>. Neonatal cholestasis (NC) poses a major diagnostic challenge to the pediatricians from an anatomical standpoint<sup>1</sup>. The focus of the initial approach is to differentiate between intrahepatic (IHC) and extrahepatic (EHC) cholestasis. Differentiation between IHC and EHC may have a success rate of 90 to 95% when various diagnostic methods are used<sup>1</sup>.

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Children with biliary atresia referred for surgery before 60 days of age do dramatically better than those older than 90 days at the time of operation in regard to re-establishment of bile flow which is more than 80% versus less than 20% respectively<sup>6</sup>. Clinical features like jaundice, pale stools and dark urine, individually, are imperfect ways of differentiating BA from INH<sup>7</sup>. There is no single test that can definitely differentiate these two entities<sup>5</sup>. For early identification of BA cases, the use of a stool color card, ultrasonography for the presence of the triangular cord sign, and also correlation of clinical, laboratory and histopathological findings have been proposed<sup>1</sup>.

The aim of this study was to evaluate the diagnostic accuracy of clinical and laboratory parameters for diagnosis of biliary atresia.

## Methods

This cross sectional study was conducted on consecutive infants having neonatal cholestasis attending the Pediatric Gastroenterology and Nutrition department of BSMMU from August 2013 through July 2015. NC is defined as the onset of clinically apparent jaundice within the first 4 months of life, with the conjugated bilirubin >1 mg/dl if the total bilirubin was <5 mg/dl, or the conjugated<sup>8</sup>. The clinical details including perinatal history were recorded. Stools were observed by one of the authors for three consecutive days to decide whether they were pigmented or pale coloured stools. Physical examination of all cases were done on the day of admission or afterwards whenever possible. The size of liver (in centimeter, measured with a non-stretchable measuring tape) was measured along the mid-clavicular line below the right costal margin. Hepatomegaly was defined as a palpable liver of >2 cm below the right costal margin along the mid-clavicular line<sup>9</sup>. The upper border of the liver was not determined because of the imprecise nature of such measurement in infants. Splenomegaly was defined as a palpable spleen of any size below the costal margin<sup>9</sup>. For all patients, serum bilirubin total and direct serum alanine

aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT), Prothrombin time (PT) were checked. Investigations were done for diagnosis of cause like infectious, metabolic and others. When any infectious or metabolic cause was found, no further work up was needed. Abdominal ultrasonography (USG) was done after 4 h of fasting and repeated ½ hour after a feed. The absence of gallbladder after fasting and lack of contraction after a meal were taken as USG evidence of BA<sup>5</sup>. Hepatobiliary scintigraphy using technetium-labeled iminodi-acetic acid analogues was to be done after giving Ursodeoxycholic acid (15 mg/kg/day orally 2 divided doses) and phenobarbitone (5 mg/Kg/day orally in two divided doses) for at least 48-72 hours. In patients in whom no intestinal tracer excretion detected even after 24 hrs, was considered as biliary atresia. After ensuring normal coagulation parameters and platelet counts, percutaneous liver biopsy was done with a true-cut liver biopsy needle under after giving local anesthesia. Informed written consent was taken from the parent before liver biopsy. Liver biopsy was interpreted by one liver pathologist who was not aware of the clinical diagnosis. Liver biopsy was done in all except those where diagnosis was otherwise obvious like infections, choledochal cysts or improving, INH with pigmented stools. For the purpose of comparison, all cases other than BA and INH were excluded. The accuracy of the diagnostic methods clinical features and liver enzymes were evaluated for differentiation Biliary atresia from idiopathic neonatal cholestasis. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of each method was calculated with liver biopsy as gold standard. The study was approved by the institutes ethical committee.

## Statistical analysis:

All the data were tabulated and calculated manually. Statistical analyses were carried out using the SPSS, version 17 (SPSS Inc., Chicago, IL, USA) for Windows XP. Results

were expressed as mean, standard deviation, range and frequency.

## Results:

During the study period a total 165 consecutive cases were initially selected as cases of neonatal cholestasis and after further diagnostic evaluations 79 cases were excluded. Among the excluded subjects 38 cases were diagnosed as neonatal hepatitis, 6 were severely ill and liver biopsy could not be done in 35 cases. Eighty six patients were selected as study subjects. Among this 86 cases 48(55.8%) were diagnosed as INH and 38(44.2%) cases as BA. There is no statistical significant difference between BA and INH in terms of sex (p 0.401)(table-2). No significant mean difference of age at admission, age at onset of jaundice and current weight were found between BA and INH cases (table 1). There exists a significant mean difference of birth weight between BA and INH (p 0.008) and mean liver size was higher in INH (5.4±1.2 cm) than that of BA (4.3±1.0 cm) (p 0.000) (table-1).

**Table-1:** Comparison of age, weight, liver size and spleen size of cases with BA and INH

Characteristics	Total (n- 86) (mean ± sd)	BA (n-38) (mean ± sd)	INH (n-48) (mean ± sd)	p <sup>a</sup> value
Age at admission (days)	85.5 ± 43.8	81.5 ± 38.4	88.7 ± 46.5	.441
Age at onset of Jaundice ( days)	6.9 ± 7.5	6.4 ± 7.7	7.5 ± 7.4	.497
Birth weight ( kg )	2.7 ± 0.4	2.9 ± 0.4	2.6 ± 0.5	0.008
Weight at admission ( kg )	4.7 ± 0.8	4.8 ± 0.7	4.7 ± 0.9	0.562
Liver size (cm)	4.8 ± 1.3	4.3 ± 1.0	5.4 ± 1.2	0.000
Spleen size (cm)	2.5 ± 1.6	2.5 ± 1.5	2.4 ± 1.6	0.968

<sup>a</sup> Independent t-test, SD - standard deviation

Most (35, 92.1%) of the infants of BA were term babies (p 0.023). Birth weight of  $\geq 2.5$  kg was found significantly more in BA (33,86.8%) cases than that of INH (p 0.004). The presence of persistent pale colored stool was more commonly seen in patients with BA (86.8%) (p 0.000). (Table -2).

**Table-2:** Comparison of Clinical characteristics of patient's between BA and NH(n=86)

Clinical variables	Total (n = 86) no (%)	BA (n = 38) no (%)	INH (n = 48) no (%)	p value
Sex				
Male	67 (77.9)	28 (73.7)	39 (81.3)	0.401 <sup>a</sup>
Female	19 (22.1)	10 (26.3)	9 (18.8)	
Gestational age				
Term	70 (81.4)	35 (92.1)	35 (72.9)	0.023 <sup>b</sup>
Preterm	16 (18.6)	3 (7.9)	13 (27.0)	
Birth weight ( kg )				
$\geq 2.5$	61 (70.9)	33 (86.8)	28 (58.3)	0.004 <sup>a</sup>
$< 2.5$	25 (29.1)	5 (13.2)	20(41.7)	
Pale color stool				
Persistent	46( 53.5 )	33 ( 86.8 )	13 ( 27.1 )	0.000 <sup>a</sup>
Intermittent	40( 46.5 )	9 ( 13.2 )	35 ( 72.9 )	
History of consanguinity				
Degree of jaundice	4 ( 4.7 )	0 ( 0.0 )	4 ( 8.3 )	0.068 <sup>b</sup>
Mild	29 ( 33.7 )	16 (42.1)	13 ( 27.1 )	0.340 <sup>a</sup>
Moderate	42 ( 48.8 )	16(42.1)	26 ( 54.2 )	
Severe	15 ( 17.5 )	6 (15.8)	9 ( 18.8 )	
Liver palpable	86 (100)	38 (100.0)	48 (100.0)	-
Spleen palpable	33 (38.4)	14 (36.8)	19 (39.6)	0.795 <sup>a</sup>
Ascites	5 (5.8)	2 (5.3)	3 (6.3)	0.846 <sup>b</sup>

<sup>a</sup>x<sup>2</sup>-test ; <sup>b</sup> Fisher's Exact Test

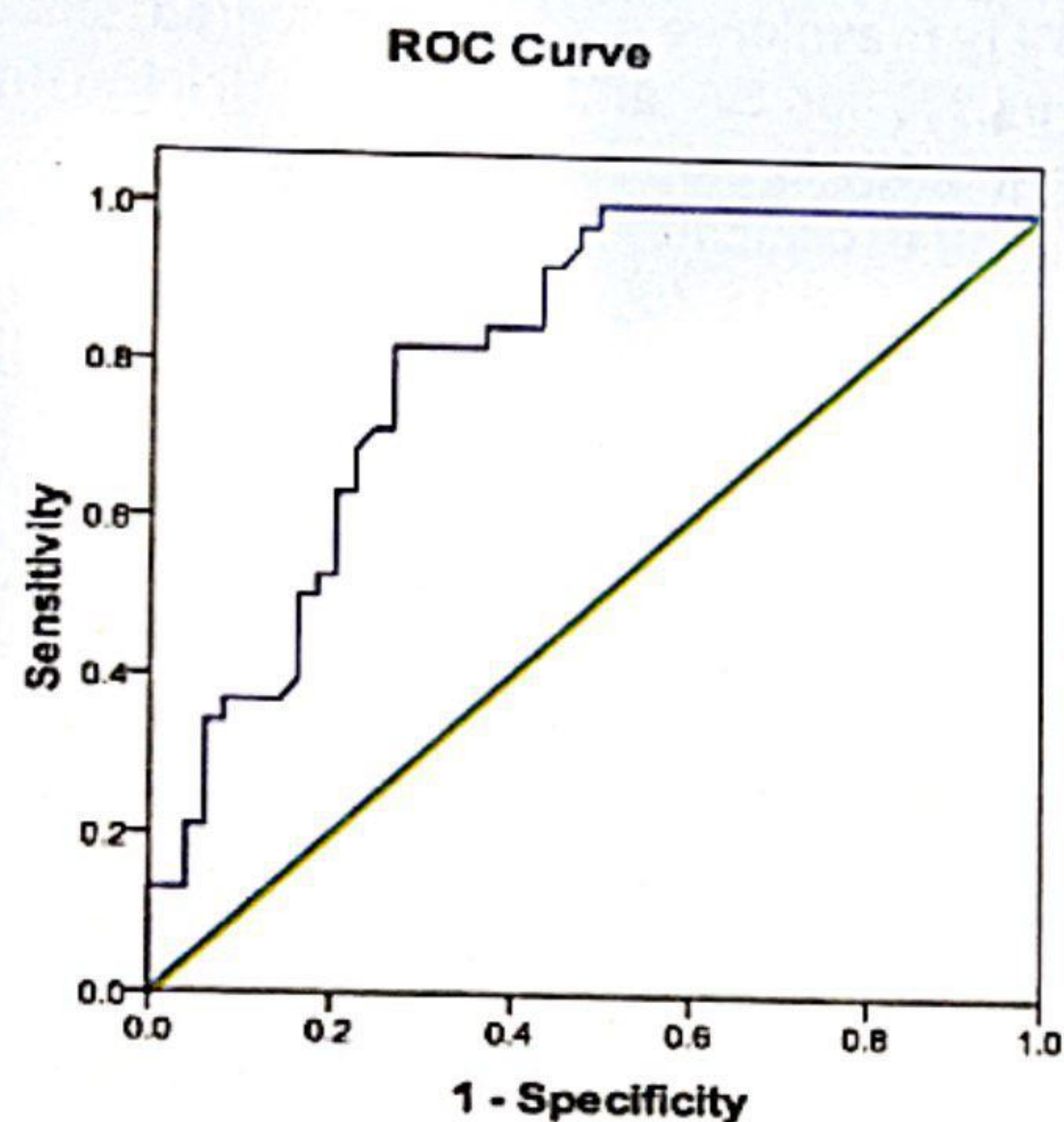
Though median AST and ALP was higher in BA (180 U/L and 563 U/L respectively) but there is no statistically significant differences. Median serum GGT was higher (921.0 U/L) in BA cases. Significant median difference of GGT was observed between BA and INH cases (p 0.000) (Table-3).

**Table-3:** Comparison of liver function tests between BA and INH cases at the time of diagnosis

Laboratory parameters	Total (n = 86)	BA (n= 38)	INH (n= 48)	p value
S. bilirubin total (mg/dl) <sup>d</sup>	12.2 ± 4.9	11.3 ± 4.5	13.0 ± 5.2	0.103 <sup>a</sup>
S. bilirubin direct (mg/dl) <sup>d</sup>	7.7 ± 3.5	7.4 ± 3.2	7.94 ± 3.7	0.494 <sup>a</sup>
ALT (IU/L) <sup>c</sup>	169.0	156.5	178.0	0.337 <sup>b</sup>
AST (IU/L) <sup>c</sup>	174.0	180.5	160.0	0.406 <sup>b</sup>
ALP (IU/L) <sup>c</sup>	541.0	563.0	412.0	0.215 <sup>b</sup>
GGT (IU/L) <sup>c</sup>	543.0	921.0	264.0	0.000 <sup>b</sup>
INR <sup>c</sup>	1.09	1.08	1.14	0.337 <sup>b</sup>

<sup>a</sup> Independent t-test ; <sup>b</sup> Mann Whitney U-test ; <sup>c</sup> Median ; <sup>d</sup> Mean ±SD; SD - standard deviation,

ROC curve analysis of GGT for diagnosis of BA showed the AUC was 0.81(p 0.000) with cut point of 524 U/L (Figure-1). The sensitivity and specificity of GGT in differentiating cases with BA and INH with cut point of 524 U/L was 81.6 % and 72.9%, respectively and diagnostic accuracy was 76.7% (Table-4).



**Figure-1:** Results of ROC curve analysis for absolute GGT values

AUC = area under curve ; GGT = gamma-glutamyl-transferase; ROC = receiver operating characteristic.

**Table-4:** Diagnostic usefulness of various clinical features and GGT for diagnosis BA

Features	Sensitivity %	Specificity %	PPV %	NPV %	Diagnostic accuracy %
Gestational age	92.1	27.1	50.0	81.3	50.1
Birth weight	86.8	31.3	50.0	75.0	55.8
Pale colored stool	86.8	72.9	71.7	87.5	79.1
GGT 524 U/L	81.6	72.9	70.5	83.3	76.7

PPV –Positive predictive value; NPV-Negative predictive value.

## Discussion

In infants with cholestatic jaundice, the major diagnostic challenge is to distinguish biliary atresia from other causes such as infectious, metabolic and idiopathic neonatal hepatitis. The diagnosis is difficult because of the several possible diagnoses with similar clinical presentation and the lack of specificity of the available diagnostic tests.

This hospital based cross sectional study was carried out to evaluate usefulness of clinical and laboratory parameters for differentiating BA from INH. The study was carried out at the department of Pediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, from August 2013 through July 2015.

During the study period, a total of 86 consecutive cholestatic cases were evaluated and their mean age at admission was  $85.52 \pm 43.76$  days and mean age at onset of jaundice was  $6.99 \pm 7.48$  days. In a previous study<sup>10</sup> conducted on similar subjects and in the same centre, the mean age at presentation of cases was 105 days while the mean age at onset of jaundice was 5.8 days and these findings are almost consistent with the findings of present study. In a consensus report<sup>11</sup> on Neonatal Cholestasis Syndrome published from India it was observed that there was a long delay by parents in seeking medical attention for their affected infants with an average duration of 4.5 weeks and the average age at presentation to a specialized center was 3.5. The Canadian Pediatric Hepatology Research Group (CPHRG) reported that 14% of Canadian infants with jaundice due to biliary atresia presented to tertiary referral centers after the age of 3 months. However, multiple cohort studies have reported that infants surgically treated for biliary atresia before 3 months of age had improved overall survival at up to 15 years of age.<sup>12,13</sup> Dehghani et al. (2006)<sup>14</sup> reported that the mean age at onset of jaundice was significantly lower in cases of biliary atresia when compared to idiopathic neonatal hepatitis cases ( $9 \pm 13$  days versus  $20 \pm 21$  days; p 0.032). But it is inconsistent with the result of present study (p 0.50).

In similar study<sup>15</sup> that examined the reasons for late referral of infants with cholestatic jaundice, it was found that either inadequate follow-up or reassurances by primary health care providers that the jaundice was physiologic was the commonest cause of late referral. However, both are preventable causes of delay. To help reduce the average age for diagnosis of biliary atresia, and hence improve outcome from the portoenterostomy procedure, several groups in Japan and Taiwan have developed pilot programs in which stool color cards are given to mothers of newborns (Hsiao et al.,2008). These cards have seven or eight numbered color photographs of age matched infant stools of varying colors, including three acholic stools. When infants are aged 1 month, the mothers were instructed to compare their infant's stool color with those printed on the card, to fill in a corresponding number, and to take the card to their 1-month physician office visit.

The present study found that mean birth weight was more in subjects having biliary atresia ( $2.87 \pm 0.42$  kg) than those of subjects having idiopathic neonatal hepatitis ( $2.61 \pm .46$  kg) and the difference is statistically significant ( $p$  0.008) and the frequency of male gender was 73.7 % and 81.3 % in BA and INH respectively.

In the present study, all subjects (100%) had history of passage of pale stool: 50 (58.1 %) had history of persistent and rest 36 (41.9 %) had intermittent passage of pale coloured stool. All subjects (100%) had hepatomegaly. Splenomegaly was found in 33 (38.4 %) studied subjects. Ascites was found in 5 (5.8 %) subjects. In a similar type study Karim & kamal (2005)<sup>10</sup> showed , history of persistent passage of pale stool was more among subjects having biliary atresia (86.8 %) than subjects having INH (27.1%) and requencey of hepatomegaly was almost similar in both groups (NH & INH: 86.5% and BA: 87.5%). Splenomegaly (73% vs 62.5%) and ascites (13.5% vs 6.2%) were more frequent among subjects having NH & INH. In the present study frequency of persistent pale coloured stool was significantly higher in BA cases(76.3

% Vs 43.8%) ( $p$  0.002). Liver size is significantly larger in INH than BA ( $5.3 \pm 1.3$  vs  $4.3 \pm 1.0$ ) ( $p$  0.000).

Dehghani et al. (2006)<sup>14</sup> studied 65 infants with neonatal cholestasis showed clinical evaluation had good accuracy for diagnosing BA (84.2%) and moderate accuracy for INH (65.2%). Prevalence's of pale coloured stool was 94.7% for BA and 56.5% for INH. The present study is consistent with their findings. Among the clinical features persistent pale coloured stool is found to be more useful (sensitivity, specificity and accuracy 86.8 %,72.9% and 79.1% respectively).

Hamid et al. (2012)<sup>16</sup> studied 40 neonatal cases in the same center and found 12 patients had BA of them 11(92%) were term and only 1 was preterm, whereas in NH group, larger number of babies 10 (56%) were preterm and 8 (44%) out of 18 babies were term babies, which was statistically significant ( $p$  <0.05). It is consistent with the present study where the frequency of term baby is significantly higher in BA 35 (92.1 %) than that of INH 72.9 % ( $p$  0.023).

Biliary atresia reported to be commoner in female infants<sup>17</sup>. However, in the present study, it was common in male (28 of 38). This is consistent with a Bangladeshi study<sup>10</sup>. This male preponderance in the present series may be due to more parental concern about their male infants.

Biliary atresia is of two types: fetal (20%) and perinatal (80%). The fetal form of biliary atresia is associated with early onset jaundice and acholic stool (within the first 3 weeks of life without jaundice free period), whereas the acquired form of biliary atresia generally has onset of jaundice and acholic stool in the 2<sup>nd</sup> to 4<sup>th</sup> weeks of life after a period of normal pigmented stool<sup>17</sup>. In this series, jaundice was noticed before two weeks in 28 of 38 infants with biliary atresia. Thus most of the cases with biliary atresia were of fetal type. Since infections are common in this region, a prenatal or intrauterine infection may have been responsible for increase number of fetal

type biliary atresia in our country though no associated congenital anomaly was detected clinically or sonologically.

It has been reported that serum bilirubin rarely exceed 12 mg/dl (may be as low as 5-8 mg/dl) in infants with biliary atresia despite complete bile duct obstruction whereas it may exceed 20 mg/dl in those with neonatal hepatitis<sup>17</sup>. In the present series, mean total S. bilirubin was  $11.3 \pm 4.5$  mg/dl in infants with biliary atresia and  $13.0 \pm 5.2$  mg/dl in infants with idiopathic neonatal hepatitis. These levels of serum bilirubin are consistent with the findings of Karim & Kamal (2005)<sup>10</sup> where they reported 10.4 mg/dl and 14.1 mg/dl in cases of biliary atresia and neonatal hepatitis respectively.

There have been various attempts to combine clinical and laboratory parameters for the differential diagnosis of BA and INH. Dehghani et al.(2006)<sup>14</sup> studied 62 infants with NC and found a prevalence of acholic stools in 94.7% of patients with BA and 56.5% of those with IHC. Results from the study by Dehghani et al.(2006)<sup>14</sup> is comparable with Poddar et al. (2009)<sup>5</sup> that studied 101 infants with neonatal cholestasis and found persistent pale coloured stool and USG have modest sensitivity and specificity in differentiating BA from INH. Onset of jaundice in the first 2 weeks of age, total serum bilirubin  $120 \mu\text{mol/L}$  and conjugated bilirubin  $68 \mu\text{mol/L}$  were highly sensitive in diagnosing BA but they are not specific for it (Poddar et al.,2009)<sup>5</sup>.In the present study persistent pale coloured stool is the only parameter that has a good accuracy (79.1%).The accuracy of persistently pale color stool in our study is slightly less than Poddar et al.,(2009)<sup>5</sup> which was 80 %.These simple clinical and laboratory parameters can be used as screening tests but to diagnose BA a test with better accuracy needs to be done.

Bellomo-Brandao et al. (2010)<sup>1</sup> studied 168 patients with neonatal cholestasis and observed that there was no significant difference of the level of liver enzymes between BA and NH group. Dehghani et al. (2006) showed that the level of liver enzymes

would not be an accurate method to differentiate BA and INH (diagnostic accuracy of 50.8%). In present study no significant difference was found in median value of ALT, AST ALP between BA and INH but median value of GGT was significantly higher in BA ( 921 U/L vs 264 U/L) (p 0.000).

GGT is an enzyme that transfers the gamma-glutamyl group from glutathione and other peptides. It is present in the biliary epithelium and hepatocytes. GGT levels are elevated in various liver diseases. GGT is also present in the kidney, breast, intestine, brain, pancreas, and spleen. Normal values vary with age, gender, and diagnostic methods. It is recognized as a useful test for differentiating familial cholestatic syndromes. The use of GGT to differentiate IHC from EHC was not recommended by Moyer et al. (2004)<sup>8</sup>, who classified it as level C evidence, because a wide variability of tests makes the interpretation of results more difficult. Lai et al.(1994)<sup>18</sup> found GGT values  $> 300 \text{ UI/L}$  with a specificity of 82.8% and accuracy of 72% for the diagnosis of EHC. Bellomo-Brandao et al. (2010)<sup>1</sup> found cutoff value  $> 429.5 \text{ UI/L}$  or 10.8 times the upper limit of normal for GGT, specificity was 91.5% and accuracy was approximately 76% for BA. Due to the higher level of GGT in biliary atresia and significant difference between BA and INH (p 0.000), ROC curve analysis was used to define probable cut points of GGT in diagnosing cases with biliary atresia (Fig. 1). AUC was 0.81(p 0.000) with cut-point of 524 U/L. In the present study the sensitivity and specificity, of GGT diagnosis of cases with BA with cut-point of 524 U/L was 81.6 % and 73 % respectively.

### Conclusion:

In the present study, among the studied clinical parameters persistent pale coloured stool was found to be the most useful to suggest the presence of biliary atresia .The study showed that serum level of GGT at cut-off vale 524 U/L may be used as a reliable laboratory parameter to differentiate biliary atresia and idiopathic neonatal hepatitis.

Further prospective studies with larger sample size may give higher sensitivity and specificity for diagnosis of biliary atresia if persistent pale coloured stool and serum level of GGT with a cut-off value 524 U/L can be correlated.

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