Original article

COMPARISON OF DIFFERENT DIAGNOSTIC METHODS IN INFANTS FOR DIFFERENTIATING IDIOPATHIC NEONATAL HEPATITIS FROM BILIARY ATRESIA

Md. Mahmudul Hasan[1], Mosfika Rahman[2], Md. Namely Hassan[3], ANM Shamsul Islam[4], Mohammad Ahmadur Rahman[5], Md. Shafiul Alam[6], Rana Kumar Biswas[7], ASM Bazlul Karim[8], Md. Rukunuzzaman[9]

ABSTRACT

Background: Biliary atresia (BA) and idiopathic neonatal hepatitis (INH) are two most common etiologies of neonatal cholestatic jaundice. It is important to distinguish INH from BA ~~biliary atresia~~ in an infant. The study was conducted to compare of different diagnostic methods in infants in differentiating INH and BA.

Methods: This cross-sectional study was conducted at the Department of Pediatric Gastroenterology & Nutrition, BSMMU during the period from July 2018 to June 2019 among infants with neonatal cholestasis. Total 50 cholestatitic cases were selected through purposive sampling. A thorough history and physical examination were done and liver enzymes were studied. Abdominal ultrasonography, hepatobiliary scintigraphy and liver biopsy were done in all cases. Clinical parameter and investigational sensitivity, specificity, and diagnostic precision were calculated. To diagnose BA and INH, liver biopsy was considered the gold standard.

Results: Among the participants, 72% were diagnosed as BA and 28% were INH. More than half of patients were male. There exists significant difference of birth weight and term baby between BA and INH. Presence of persistent pale coloured stool was more commonly seen in patients with BA. Diagnostic accuracy of persistent pale coloured stool, birth weight (normal) and term baby were 94%, 92% & 88% respectively. Sensitivity and specificity of Gamma-glutamyl transpeptidase (GGT) in differentiating cases with BA and INH with cut point of ≥430.0 U/L were 86.1% and 85.7%, respectively. Hepatobiliary scintigraphy is found to have a better sensitivity than ultrasonography. GGT had diagnostic accuracy 86%, hepatobiliary scintigraphy had diagnostic accuracy 88% & USG had diagnostic accuracy 80% for BA and INH.

Conclusion: Among the studied clinical parameters persistent pale coloured stool was most accurate to suggest the presence of BA. Serum level of GGT at cut-off value ≥ 430 U/L may be used as a reliable laboratory parameter to differentiate INH and BA. Hepatobiliary scintigraphy found to have a good sensitivity for BA. Positive result of hepatobiliary scintigraphy may be used as BA in infants with cholestasis.

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1. Assistant Professor, Department of PGN, SZMC, Bogura
2. Assistant Registrar, Medical oncology, NIRCH, Dhaka
3. Registrar, Department of Pediatrics, SZMC, Bogura
4. Associate Professor, NIPSOM, Dhaka
5. Assistant Professor, Department of PGN, SHMC, Jamalpur
6. Assistant Professor, Department of PGN, ShSMC, Dhaka
7. Assistant Professor, Department of PGN, KMC, Khulna
8. Ex-chairman, Department of PGN BSMMU, Dhaka
9. Chairman, Department of PGN BSMMU, Dhaka

Correspondence: *Md. Mahmudul Hasan; Email: mahmud.paed@gmail.com*

INTRODUCTION

Cholestasis can be characterized physiologically as a measurable reduction in bile flow, pathologically as the histologic presence of bile pigment in hepatocytes and bile ducts, and clinically as the accumulation of substances typically excreted in bile (such as bilirubin, bile acid, and cholesterol) in the blood and

extrahepatic tissues. The process occurs as a result of impaired bile formation by the hepatocyte or from obstruction to the flow of bile through intrahepatic and extrahepatic biliary tree.[1-2] It is characterized by direct bilirubin value is greater than 1.0 mg/dl, the total bilirubin is less than 5mg/dl or direct bilirubin of >20% of the total, if the total bilirubin is greater than 5mg/dl.[3] The prevalence of newborn liver disease as a whole, with the majority of cases displaying clinical or biochemical indications of cholestasis, may be as high as 1 in 2500 live births.[4] Jaundice is the most overt physical sign of liver disease and occurs more commonly in the neonatal period than at any other time of life.[5] Any neonate with jaundice who is more than two weeks' old has to be evaluated for liver or biliary tract illness. These infants should be evaluated for cholestasis by measurement of total and conjugated serum bilirubin.[6]

Infants with neonatal cholestasis usually present with variable degrees of jaundice, dark urine, Pale stool and enlarged liver, all of which are nonspecific features of neonatal cholestasis. Biliary atresia and idiopathic neonatal hepatitis are two most common etiologies of neonatal cholestatic jaundice.[7] It is important to distinguish idiopathic neonatal hepatitis from biliary atresia in an infant presented with jaundice, as the former purely needs a medical management and the later requires surgical intervention as soon as possible. Therefore, rapid and accurate differentiation is crucial for early surgery in patients with biliary atresia.[8] Biliary atresia is the most common single indication for liver transplantation in children.[9] The tests that have been used to make this differentiation: clinical evaluation, liver enzymes, USG, HIDA and liver biopsy. It will help us to compare of different diagnostic method. So, the objective of this study is to evaluate and compare different diagnostic methods in differentiating INH and biliary atresia.

METHODS

This cross sectional study was carried out at the department of pediatric gastroenterology and nutrition of BSMMU, Shahbag, Dhaka, Bangladesh from July 2018 to June 2019. The study was conducted on consecutive infants having neonatal cholestasis. By method of exclusion 50 cases were included in this study. A standard questionnaire was designed with a view to collect data from the patients/respondents. Before collection of data, institutional permission was taken. The detail clinical history, physical examination findings and investigation reports were recorded in the preformed standard data sheet by the researcher. Every ethical issue was discussed with the patient's parents regarding the study and informed written consent was obtained. Statistical analyses were carried out using the SPSS.

RESULTS

Distribution of studied subjects

The study found that Fifty patients were selected as study subjects. Among this 50 cases 28% were diagnosed as INH and 72% as BA cases (Figure 1).

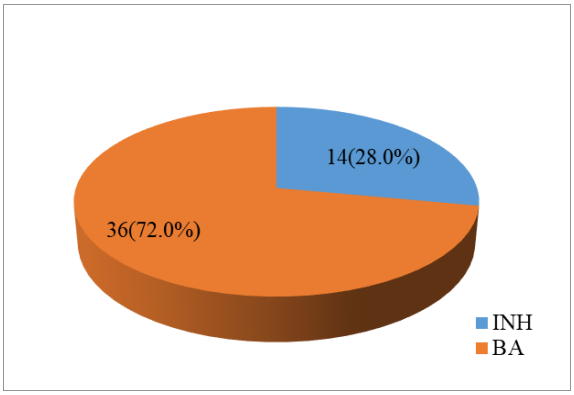

Figure 1. Pie chart showing the distribution of cases (n=50)

It was revealed that most (91.7%) of the infants of BA were term babies while 78.6% cases were preterm baby in INH is statistically significant. Birth weight of ≥ 2.5 kg was found significantly more in baby with BA than that of INH. The presence of persistent pale coloured stool was more commonly seen in patients with BA. The difference is statistically significant (Table 1).

Table 1. Clinical symptoms differentiating BA from INH (n=50)

	Total (n=50)		BA (n=36)		INH (n=14)		p value
	N	%	n	%	N	%	
History of consanguinity							
Present	3	6.0	1	2.8	2	14.3	0.186[ns]
Absent	47	94.0	35	97.2	12	85.7	
Gestational age							
Preterm	14	28.0	3	8.3	11	78.6	0.001[s]
Term	36	72.0	33	91.7	3	21.4	
Birth weight (kg)							
<2.5	12	24.0	1	2.8	11	78.6	0.001[s]
≥2.5	38	76.0	35	97.2	3	21.4	
Pale color stool							
Persistent	33	66.0	33	91.7	0	0.0	0.001[s]
Intermittent	17	34.0	3	8.3	14	100.0	

s= p<0.05, significant at 95% CI; ns= not significant

It was found that mean difference of serum total bilirubin and direct bilirubin was not statistically significant between BA and INH. The difference in mean ALT, AST, ALP and INR values between BA and INH was not significant. Mean serum GGT was significantly higher in BA than INH (Table 2).

Table 2. Comparison of liver function tests between BA and INH cases at the time of diagnosis (n=50)

	Total (n=50)	BA (n=36)	INH (n=14)	p value
	Mean±SD	Mean±SD	Mean±SD	
S. Bilirubin total (mg/dl)	10.42±4.21	10.65±4.41	9.82±3.71	0.538[ns]
S. Bilirubin direct (mg/dl)	6.56±3.42	6.64±3.55	6.36±3.17	0.800[ns]
ALT (U/L)	155.0±101.2	154.3±104.9	156.9±94.4	0.936[ns]
AST (U/L)	241.0±134.3	263.9±114.2	182.1±166.4	0.052[ns]
ALP (U/L)	518.3±289.7	562.5±326.4	404.7±101.1	0.084[ns]
GGT (U/L)	749.9±544.9	934.6±500.5	328.0±275.0	0.001[s]
INR	1.28±0.75	1.28±0.80	1.28±0.62	0.988[ns]

s= p<0.05, significant at 95% CI; ns= not significant

The study showed that contracted gallbladder was found in 18(50.0%) with BA and 3 (21.4%) in INH cases and the difference was significant. Contraction of gallbladder was statistically significant between two groups. An ultrasonic diagnosis of BA was made in 36 (72.0%) infants and that of INH in 14 (28.0%) infants (Table 3).

Table 3. Comparison of USG findings between BA and INH (n=50)

	Total (n=50)		BA (n=36)		INH (n=14)		p value
	N	%	N	%	N	%	
Gallbladder							
Normal	20	40.0	10	27.8	10	71.4	
Absent	9	18.0	8	22.2	1	7.1	[a]0.018[s]
Contracted	21	42.0	18	50.0	3	21.4	
Contractility							
Normal	22	44.0	9	25.0	13	92.9	[b]0.001[s]
Absent	28	56.0	27	75.0	1	7.1	
Hepatomegaly	50	100.0	36	100.0	14	100.0	-
Splenomegaly	22	44.0	14	38.8	6	42.8	[a]0.780[ns]

Ascites							
Mild	1	2.0	1	2.8	0	0.0	[a]0.667[ns]
Moderate	1	2.0	1	2.8	0	0.0	
Absent	48	96.0	34	94.4	14	100.0	
Triangular cord sign							
Present	2	4.0	2	5.6	0	0.0	[b]0.514[ns]
Absent	48	96.0	34	94.4	14	100.0	
USG diagnosis							
BA	36	72.0	31	86.1	5	35.7	[a]0.001[s]
INH	14	28.0	5	13.9	9	64.3	

The study found that good uptake of radiotracer was found 32(88.9%) in BA and 3(21.4%) in INH that was statistically significant. Excretion of the radiotracer into the intestine was present 3(8.3%) and 11(78.6%) in BA and INH respectively, which was statistically significant between two groups. Scintigraphy was significantly positive among BA (Table 4).

Table 4. Scintigraphic findings in the studied subjects (n=50)

Scintigraphic findings	Total (n=50)		BA (n=36)		INH (n=14)		p value
	N	%	n	%	N	%	
Uptake of the radiotracer (liver within 30 min of scan)							
Good	35	70.0	32	88.9	3	21.4	0.001[s]
Poor	15	30.0	4	11.1	11	78.6	
Excretion of the radiotracer into the intestine (within 24 hour)							
Present	14	28.0	3	8.3	11	78.6	0.001[s]
Absent	36	72.0	33	91.7	3	21.4	
Scintigraphic diagnosis							
BA	36	72.0	33	91.7	3	21.4	0.001[s]
INH	14	28.0	3	8.3	11	78.6	

s= $p<0.05$, significant at 95% CI; ns= not significant

Table 5 shows that preserved hepatic architecture was significantly higher in BA than INH. Giant cell was 1(2.8%) and 13(92.9%) in BA and INH group respectively that was significantly higher in INH than BA. Bile ductular proliferation, bile plugs and portal cellular infiltration were significantly higher in BA than in INH cases.

Table 5. Comparison of liver biopsy findings between BA and INH (n=50)

Liver biopsy findings	Total (n=50)		BA (n=36)		INH (n=14)		p value
	N	%	n	%	N	%	
Hepatic architecture							
Preserved	43	86.0	36	100.0	7	50.0	0.001[s]
Disturbed	7	14.0	0	0.0	7	50.0	
Giant cell							
Present	14	28.0	1	2.8	13	92.9	0.001[s]
Absent	36	72.0	35	97.2	1	7.1	
Bile ductular proliferation							
Present	35	70.0	34	94.4	1	7.1	0.001[s]
Absent	15	30.0	2	5.6	13	92.9	

Bile plug							
Present	32	64.0	31	86.1	1	7.1	0.001[s]
Absent	18	36.0	5	13.9	13	92.9	
Portal fibrosis							
Present	36	72.0	26	72.2	10	71.4	0.606[ns]
Absent	14	28.0	10	27.8	4	28.6	
Portal cellular infiltration							
Present	40	80.0	26	72.2	14	100.0	0.025[s]
Absent	10	20.0	10	27.8	0	0.0	
Cirrhosis							
Present	3	6.0	3	8.3	0	0.0	0.364[ns]
Absent	47	94.0	33	91.7	14	100.0	

s= significant, ns= not significant

Table 6 shows that scintigraphy had sensitivity and specificity of 91.7% and 78.6%, respectively. USG had sensitivity 86.1%, specificity 64.3% for detecting BA. Scintigraphy had sensitivity and specificity of 91.7% and 78.6%, respectively.

Table 6. Sensitivity and specificity of various diagnostic methods for BA and INH

Diagnostic method	Sensitivity for BA		Specificity for BA	
	%	n	%	N
GGT ≥430.0 U/L	86.1	31/36	85.7	12/14
USG	86.1	31/36	64.3	9/14
Hepatobiliary scintigraphy	91.7	33/36	78.6	11/14

Table 7 reveal that GGT ≥430.0 U/L had 86% diagnostic accuracy for BA and INH. USG had 80% diagnostic accuracy for BA and INH and hepatobiliary scintigraphy had diagnostic accuracy 88% for BA and INH.

Table 7. Accuracy of various diagnostic methods for BA and INH

Diagnostic method	BA	INH	INH & BA accuracy	
	n	n	n	%
GGT ≥430.0 U/L	31/36	12/14	43/50	86.0
USG	31/36	9/14	40/50	80.0
Hepatobiliary scintigraphy	33/36	11/14	44/50	88.0

DISCUSSION

Infants with cholestatic jaundice, diagnosis is difficult because of the several possible diagnoses with similar clinical presentation and the lack of specificity of the available diagnostic tests. The present study found that mean birth weight was more in subjects having biliary atresia (2.87 ± 0.26 kg) than those of subjects having idiopathic neonatal hepatitis (2.25 ± 0.30 kg) and the difference is statistically significant (p 0.001). All subjects (100%) had history of passage of pale stool: 33 (66.0%) had history of persistent and rest 17 (34%) had intermittent passage of pale coloured stool. All subjects (100%) had hepatomegaly. Splenomegaly was found in 22 (44%) studied subjects. Ascites was found in 4 (8%) subjects. In a related study, participants with biliary atresia (86.2%) had a higher prevalence of a history of persistent transit of pale stool than participants with INH (27.1%). However, having passage of intermittent pale stool was found more frequently among subjects having neonatal hepatitis (NH) & INH (32.2% vs 18.8%). Frequency 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.[10]

In the present study, the presence of persistent pale coloured stool was more commonly seen in patients with BA. The difference is statistically significant. Liver size was larger in INH than BA (5.1 ± 1.5 vs 4.8 ± 1.4). Dehghani studied 65 infants with neonatal cholestasis showed clinical evaluation had good accuracy for diagnosing BA (84.2%) and moderate

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accuracy for INH (65.2%). Prevalence's of pale coloured stool was 94.7% for BA and 56.5% for INH.[11] 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 91.7%, 100% and 94% respectively. Another 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 ($p < 0.05$).[12] It is consistent with the present study where most (33, 91.7%) of the infants of BA were term babies while 78.6 % cases were preterm baby in INH is statistically significant.

In the present series, mean total S. bilirubin was 10.65 ± 4.41 mg/dl in infants with biliary atresia and 9.82 ± 3.71 mg/dl in infants with idiopathic neonatal hepatitis. These levels of serum bilirubin are consistent with the findings of studied where they reported 10.4 mg/dl in BA and 14.1 mg/dl in cases of neonatal hepatitis[13] that is not consistent. Bellomo-Brandao 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.[13] Dehghani showed that the level of liver enzymes would not be an accurate method to differentiate BA and INH (diagnostic accuracy of 50.8%).[11] In present study median value of GGT was significantly higher in BA (934.6±500.5 U/L vs 328±275 U/L). For GGT, Bellomo-Brandao discovered a cutoff value >429.5 UI/L, or 10.8 times the upper limit of normal; the accuracy for BA was roughly 76%, with a specificity of 91.5%.[13] Sensitivity and specificity, of GGT in differentiating cases with BA and INH with cut-point of ≥ 430 U/L were 86.1 % and 85.7 % respectively in this study.

In the present study 21 (42%) had contracted gallbladder and 9 (18%) had non visualized gallbladder and this is significantly higher in BA. Tam studied sonographic findings in 38 cholestatic patients & found 23 (61%) abnormal studies of which 15 (39%) had small gallbladder and 8 (21%) non-visualized gallbladder. However, no definite periportal fibrosis was identified in this group of patients. Predictive values for biliary atresia were 86%, 45%, 27% and 93%, respectively[14]. The present study found sensitivity and specificity of ultrasonography 86.1 % and 64.3% respectively and diagnostic accuracy 80.0% which is higher than that reported by Tam diagnostic accuracy 53% and 69.2% by Dehghani.[11]

Tam found the sensitivity and specificity of scintigraphy were 100% and 85% respectively and the overall diagnostic accuracy was 88%.[14] In the present study the sensitivity, specificity values of scintigraphy were 91.7%, 78.6 % respectively and the diagnostic accuracy was 88%. The specificity of HBS improved significantly from 63% to 86% after UDCA therapy ($p<0.001$).[15]

Liver biopsy is the most invasive method among the various tests. But it is also the most accurate one, with highest accuracy rate of 97%.[16] A retrospective study showed that biliary atresia was the most frequent biopsy-driven diagnosis, found in 58.2% cases, followed by intrahepatic bile duct paucity found in 10.9% cases.[17] In the present study, liver biopsy was done in all 50 cases and used as the gold standard for diagnosis of BA and comparison of efficacy of other diagnostic methods. The present study was able to differentiate all cases of BA from INH by liver biopsy. The accuracy of liver biopsy was 100%. Bile ductular proliferation, bile plugs and portal fibrosis, are significantly higher in BA. Giant cell transformation and portal cellular infiltration is significantly higher in INH than in BA. Thus among all the histological parameters analyzed, ductular proliferation, portal fibrosis, bile plugs and portal cellular infiltrations were the most important in distinguishing BA from INH.

CONCLUSION

The current investigation indicated that of the clinical indicators examined, persistent pale-colored stool was the most reliable indicator of biliary atresia. The study demonstrated that a reliable laboratory indicator to distinguish between idiopathic newborn hepatitis and biliary atresia is a blood level of GGT at a cut-off value of ≥ 430 U/L. Hepatobiliary scintigraphy was discovered to have a good sensitivity and positive predictive value for biliary atresia in the current investigation. Therefore, in babies with cholestasis, a positive hepatobiliary scintigraphy result may be interpreted as biliary atresia.

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