Direct Bilirubin and Risk of Biliary Atresia

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abstract

BACKGROUND: Early detection of biliary atresia (BA) is important for optimal treatment. Direct bilirubin (D-bil) levels are used for BA screening. In this study, we aimed to determine the sensitivity and specificity of elevated D-bil and the direct-to-total bilirubin (D/T) ratio for BA detection in high-risk infants.

METHODS: This retrospective, cross-sectional study was conducted in a tertiary medical center in Taiwan. Infants indicated for total bilirubin and D-bil measurements before age 60 days were included. The first bilirubin assessment was considered the test point. BA diagnosis was based on *International Classification of Diseases, Ninth and Tenth Revision*, codes 751.61 and Q44.0 to Q44.3, respectively.

RESULTS: Between January 2009 and December 2016, 4468 infants were enrolled, including 38 with BA. Among infants aged 3 to 60 days, a sensitivity of 100% (95% confidence interval, 90.3–100.0) was found for D-bil ≥1.0 mg/dL and either D-bil ≥1.0 mg/dL or D/T ratio ≥20%. However, D-bil ≥1.0 mg/dL had higher specificity (77.3% [76.0–78.5] vs 68.3% [66.8–69.7], respectively). In newborns aged <3 days, D-bil ≥0.5 mg/dL was considered a positive result, with a sensitivity of 50%. D-bil >0.45 mg/dL was a better cutoff point in receiver operating characteristic analysis, with a sensitivity and specificity of 100% (95% CI: 15.8–100) and 15.4% (95% CI, 11.8–19.7), respectively.

CONCLUSIONS: D-bil ≥1.0 mg/dL was better for BA detection than the D/T ratio in infants aged 3 to 60 days. For newborns aged <3 days, a more definitive cutoff point is required.

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WHAT'S KNOWN ON THIS SUBJECT: Patients with biliary atresia have elevated direct or conjugated bilirubin levels shortly after birth, suggesting their potential for earlier biliary atresia detection.

WHAT THIS STUDY ADDS: Direct bilirubin (D-bil) ≥1.0 mg/dL was optimal in infants aged 3 to 60 days and is preferable to the direct-to-total bilirubin ratio. For newborns aged <3 days, D-bil >0.45 mg/dL is the acceptable cutoff point.

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Cholestatic jaundice affects ~1 in every 2500 to 5000 infants.[1–3] In the joint guidelines of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition, abnormal direct bilirubin (D-bil) is defined as a serum level >1.0 mg/dL (17 mmol/L) in the presence of elevated total bilirubin (T-bil).[4] Previously, a direct-to-total bilirubin (D/T) ratio >20% was also considered to indicate cholestasis.[5]

Most cases of cholestasis in infancy can be classified as extrahepatic or intrahepatic cholestasis (Supplemental Table 4). Biliary atresia (BA) is among the most common causes of severe cholestatic liver disease in neonates and is the most common indication for pediatric liver transplantation. The incidence is ~1 per 8000 to 15 000 live births worldwide[2,6] but is higher in Asia, including Taiwan (1.48–3.7 per 10 000 live births).[7–9] In 1959, Kasai first described hepatoportoenterostomy (Kasai portoenterostomy) in patients with BA and suggested that early diagnosis and Kasai portoenterostomy before age 60 days could improve prognosis.[10] Various studies have demonstrated the benefits of early Kasai portoenterostomy (before age 30 days).[11–13]

Many strategies have been developed for early detection of BA,[14] including stool color card screening. This screening method was first used in Japan in 1994[15] and subsequently in Taiwan in 2002.[7] In Taiwan, the universal stool color card screening program for BA significantly increased the rate of Kasai portoenterostomies before age 60 days and the 5-year jaundice-free survival rate with the native liver.[8,16] However, not all patients with BA have an abnormal stool color in the early period, and some cases may be missed. In 1995, Mowat et al[17] suggested D-bil or conjugated bilirubin measurement as an alternative. Patients with BA have elevated D-bil or conjugated bilirubin shortly after birth,[18,19] which may allow prompt diagnosis. Recent studies have demonstrated good sensitivity and specificity of D-bil and conjugated bilirubin levels for BA diagnosis.[19–21]

The aim of this study, which we conducted over an 8-year period, was to determine the sensitivity and specificity of D-bil levels and the D/T ratio for BA detection on the basis of age.

METHODS

This study was based on the Integrated Medical Database of National Taiwan University Hospital, Department of Medical Research. The study protocol was approved by the institutional review board and ethics committee of National Taiwan University Hospital.

Patient Enrollment

All infants aged 0 to 60 days born in (or referred from other hospitals or clinics to) a tertiary medical center in north Taiwan between January 2009 and December 2016 with an indication for T-bil and D-bil assessment were included. Blood sampling date, T-bil, and D-bil data were recorded. Cholestasis was diagnosed as D-bil ≥1.0 mg/dL or D/T ratio ≥20%.[4,5]

The criteria for T-bil and D-bil assessment included a prenatal diagnosis of hepatobiliary disease, suspected inborn errors of metabolism or congenital infection, early-onset hyperbilirubinemia (aged <48 h), need for intensive phototherapy, rehospitalization for phototherapy for jaundice, symptoms of cholestasis or hepatobiliary disease (including abnormal stool color), prolonged total parenteral nutrition (TPN) (eg, in premature infants and cases of major surgery), and prolonged jaundice after age 2 weeks or jaundice at the time of 1-month vaccinations.

Definition and Testing for Cholestasis

The enrolled infants were divided into newborn and infant groups on the basis of age at the time of testing. The newborn group included infants aged <3 days, whereas the infant group included those aged 3 to 60 days. For both groups, the testing age was defined as the time of initial collection of T-bil and D-bil data, on which the sensitivity and specificity calculations were based. Subsequent T-bil and D-bil data were also collected, if available.

In the infant group, a positive test was defined as D-bil ≥1.0 mg/dL or D/T ratio ≥20%. Four methods were used to assess the outcomes: only D-bil ≥1.0 mg/dL, only D/T ratio ≥20%, both D-bil ≥1.0 mg/dL and D/T ratio ≥20%, and either D-bil ≥1.0 mg/dL or D/T ratio ≥20%. However, there was no clearly defined upper limit for the newborn group, and the normal ranges varied among laboratories. Therefore, a positive result was defined as D-bil ≥0.5 mg/dL on the basis of a study by Harpavat et al,[18] who used this as the cutoff point for newborns within the first 72 hours of life.

BA Diagnosis

International Classification of Diseases, Ninth and Tenth Revision (ICD-9 and ICD-10, respectively), codes were obtained for the infants before age 1 year. BA was diagnosed if the infants had BA-related ICD-9 code 751.61 and ICD-10 codes Q44.0 to Q44.3. To prevent miscoding, chart reviews of all infants with BA-related ICD codes were performed. All patients with BA

underwent Kasai portoenterostomy with liver pathology confirmation. The admission notes were checked for reports of stool color. We also identified infants with ICD-9 and ICD-10 codes for other cholestatic liver diseases (Supplemental Table 5).

Outcomes

The basic data of the infants diagnosed with BA, including age at testing, sex, gestational age, and stool color, were collected. Outcomes of interest were age at the time of Kasai portoenterostomy, jaundice-free rate at 3 and 6 months postoperatively, 2- and 5-year survival rates with native liver, and overall survival rates. Jaundice-free status was defined as T-bil <2.0 mg/dL.

Statistical Analysis

MedCalc version 20.022 software (MedCalc Software, Ostend, Belgium) was used for statistical analyses. Receiver operating characteristic (ROC) curve analysis was used to evaluate the sensitivity and specificity of D-bil and D/T ratio for BA detection and to determine a cutoff D-bil value. Positive predictive value (PPV) was defined as the number of infants with BA who tested positive divided by the total number of infants who tested positive. Negative predictive value (NPV) was defined as the number of infants without BA who tested negative divided by the total number of infants who tested negative. One-way analysis of variance was used for a comparison of diagnoses. $P < .05$ was considered statistically significant.

RESULTS

Between January 2009 and December 2016, 4468 infants aged <60 days underwent T-bil and D-bil measurements. The female:male ratio was 1:1.3. The mean age at testing was 22 ± 15.7 days; 346 infants (7.7%) were tested before age 3 days, whereas 4122 (92.3%) were tested between ages 3 and 60 days (Fig 1). In the newborn group, 271 infants tested positive, whereas in the infant group, 1333 tested positive, including 533 (40.0%) with a D-bil level ≥1.0 mg/dL, 368 (27.6%) with D/T ratio ≥20%, and 432 (32.4%) with both.

Among the enrolled infants, 334 had confirmed cholestatic liver disease according to the ICD codes, including 38 patients with BA (Table 1). Forty-seven infants had multiple cholestatic liver disease diagnoses, with TPN cholestasis being the most frequent comorbidity.

Basic Characteristics of the Patients With BA

Five patients with BA were born in our hospital, for an incidence of 1.81 cases per 10 000 live births (5 of 27 648). Among the 38 patients with BA (Table 2), 25 (65%) were girls, and the female:male ratio was 1:0.5. Six patients (15.8%) were premature, with gestational ages of 32 to 36 weeks. The median age at testing was 25 days. The median D-bil and D/T ratio were 3.99 mg/dL and 57.3%, respectively. In total, 31 (81.6%) of the 38 patients with BA had an abnormal stool color before Kasai portoenterostomy, and 37 had a D-bil level ≥1.0 mg/dL or D/T ratio ≥20% at the time of testing.

Comparison of BA Testing Methods

Newborn Group

The newborn group included 346 infants, of whom 243 (70.2%) were born in our hospital. Infants with a D-bil level ≥0.5 mg/dL were considered positive (Supplemental Table 6). The sensitivity, specificity, PPV, and NPV were 50% (95% confidence interval [CI], 1.3–98.7), 21.5% (95% CI, 17.3–26.2), 0.4% (95% CI, 0.1–1.5), and 98.7% (95% CI, 94.8–99.7), respectively. Two patients with BA had D-bil data before age 3 days, but 1 of them was not identified at the testing point. ROC curve analysis was performed to obtain an optimal D-bil cutoff point for this age (Fig 2). When the D-bil level was >0.45 mg/dL, the sensitivity and specificity for BA detection were 100% (95% CI, 15.8–100) and 15.4% (95% CI, 11.8–19.7), respectively, for infants aged <3 days.

The majority of infants who underwent D-bil assessment at this age had D-bil ≥0.5 mg/dL, exhibited

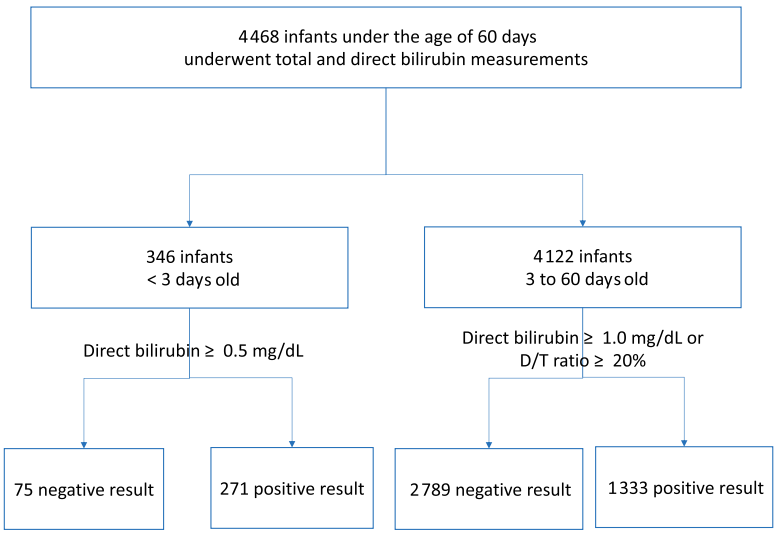

FIGURE 1

Patient flowchart. The enrolled infants were divided into 2 groups on the basis of age at testing. The newborn group included infants with a testing age <3 days, and the infant group included those with a testing age of 3 to 60 days.

TABLE 1 Distribution of Patients With Cholestatic Liver Disease

Disease Type	No.[a]
TPN cholestasis	83
Neonatal hepatitis (including CMV hepatitis)	68
Heart disease and shock-related cholestatic liver disease	60
BA	38
Inborn errors of metabolism	36
Infection related (including sepsis, UTI)	34
Choledochal cyst	14
Down syndrome	10
Paucity of intrahepatic bile duct (including Alagille syndrome)	6
Congenital hypothyroidism	5
NICCD	5
Fulminant hepatitis	4
Inspissated bile syndrome	4
Progressive family intrahepatic cholestasis	3
Congenital cystic disease of liver (ADPKD)	1
Inborn error of bile acid synthesis	1
Other	12

Related ICD-9 and ICD-10 codes are listed in Supplemental Table 5. ADPKD, autosomal-dominant polycystic kidney disease; CMV, cytomegalovirus; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; UTI, urinary tract infection.

[a] Forty-seven infants had multiple cholestatic liver disease diagnoses.

early-onset hyperbilirubinemia, and required intensive phototherapy. In total, 20 (5.8%) of the 346 infants were diagnosed with other cholestatic liver diseases, including 6 with neonatal hepatitis with or without congenital cytomegalovirus infection, 6 with TPN cholestasis, 4 with complex heart disease with or without shock-related cholestatic liver disease (1 of whom had TPN cholestasis), 2 with sepsis, 1 with hydrops fetalis with shock, 1 with hepatic hemangioma, and 1 with vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula, renal anomalies, and limb abnormalities association with choledochal cyst.

Infant Group

The infant group included 4122 infants. We used 4 different assessment methods (Supplemental Table 7). Methods 1 (D-bil ≥1.0 mg/dL) and 4 (D-bil ≥1.0 mg/dL or D/T ratio ≥20%) had the best sensitivity (100%; 95% CI, 90.3–100.0) and NPV (100%), whereas method 1 had higher specificity than method 4 (77.3% vs 68.3% [95% CI, 76.0–78.5 vs 66.8–69.7, respectively). The sensitivities of methods 2 (D/T ratio ≥20%) and 3 (D-bil ≥1.0 mg/dL and D/T ratio ≥20%) were both 94.4% (95% CI, 81.3–99.3 vs 81.3–99.3, respectively), whereas the specificities were 81.3% (95% CI, 80.0–82.4) and 90.3% (95% CI, 89.3–91.2), respectively. The ROC curves for D-bil and
D/T ratio are compared in Fig 3; *P* was .0008 (95% CI, 0.0139–0.0529).

Using method 1 (D-bil ≥1.0 mg/dL), we calculated the sensitivity and specificity for 3 age groups: 3 to 14, 15 to 30, and 31 to 60 days. The sensitivity and NPV were 100% for all groups, whereas the specificities were 74.7% (95% CI, 72.5–76.9), 69.8% (95% CI, 66.4–73.0), and 82.8% (95% CI, 80.9–84.5), respectively (Supplemental Table 8). The specificity was highest for infants aged 31 to 60 days.

In total, 965 infants had an initial D-bil ≥1.0 mg/dL, and 225 were diagnosed with cholestatic liver disease, including BA. Of the remaining 740 infants, 443 had no further D-bil data or were lost to follow-up, 167 had D-bil <1.0 mg/dL at follow-up, 46 showed a decreasing D-bil trend, and 84 showed increased D-bil levels during the follow-up, with the final diagnosis being undetermined. We compared the testing age and laboratory data between BA and 6 other cholestatic liver diseases: TPN cholestasis, neonatal hepatitis, heart disease and shock-related cholestatic liver disease, infectious diseases, Alagille syndrome, and neonatal intrahepatic cholestasis caused by citrin deficiency (Table 3). Differential diagnosis of these diseases is difficult in early infancy. There were no significant differences in testing age, T-bil, or D-bil between BA and the other diseases. However, there was a significant difference in the D/T ratio between patients with BA and heart disease and shock-related cholestatic liver disease.

Outcomes in Patients With BA

The median age for Kasai portoenterostomy in patients with BA was 45 days (range, 15–63 days). Sixteen patients with BA (42.1%) underwent liver transplantation during the study period. The 3- and 6-month jaundice-free rates after Kasai portoenterostomy were 52.6% and 55.3%, respectively. The 2- and

TABLE 2 Characteristics of Patients With BA

Characteristic	Infant Group (Aged 3–60 d, *n* = 36)	Newborn Group (Aged <3 d, *n* = 2)	Total (*N* = 38)
Female:male ratio	1:0.5	2:0	1:0.5
Prematurity, No. (%)	6 (16.7)	0	6 (15.8)
Abnormal stool color, No. (%)	30 (83.3)	0	30 (78.9)
Age of testing, range, d	3–58	1–2	25.5[a]
D-bil, range, mg/dL	1.41–8.23	0.46–1.42	3.99[a]
D/T ratio, range, %	13.2–77.0	5.9–14.8	57.3[a]
Age at Kasai portoenterostomy, d	19–63	15–41	45.5[a]

[a] Median.

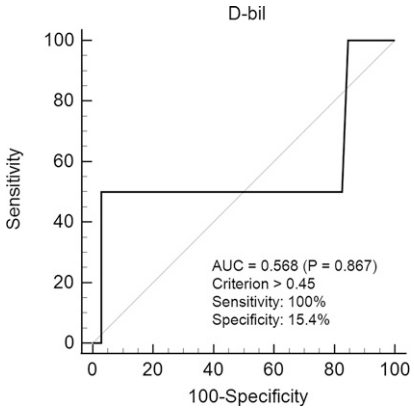

FIGURE 2

ROC curve of D-bil for BA in the newborn group (aged <3 days). The area under the curve (AUC) was 0.568 (95% CI, 0.514–0.620). When D-bil levels were >0.45 mg/dL, the sensitivity and specificity for detecting BA were 100% (95% CI, 15.8–100) and 15.4% (95% CI, 11.8–19.7), respectively.

5-year survival rates with native livers were 54.3% and 45.2%, respectively. The overall 2- and 5-year survival rates were 91.4% and 90.2%, respectively. Only 1 patient with BA did not undergo Kasai portoenterostomy before age 60 days during the study period because she was transferred to our hospital at age 56 days.

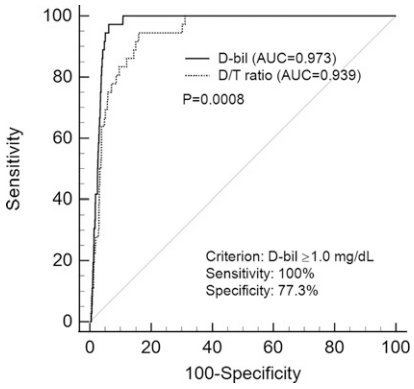

FIGURE 3

ROC curve comparison of D-bil and D/T ratio in the infant group (aged 3 to 60 days). Areas under the curve (AUC) were 0.973 (95% CI, 0.967–0.977) and 0.939 (95% CI, 0.931–0.946) for D-bil and D/T ratio, respectively. *P* was .0008 (95% CI, 0.0139–0.0529) for detecting BA. When D-bil levels were ≥1.0 mg/dL, the sensitivity and specificity for detecting BA were 100% (95% CI, 90.3–100.0) and 77.3% (95% CI, 76.0–78.5), respectively.

DISCUSSION

Early identification of patients with BA and early referral for surgery have been a challenge for pediatricians. An increase in parent and physician awareness followed the launch of the universal screening program for BA (using infant stool color cards) in Taiwan in 2004. Although the use of stool color cards for mass BA screening has been implemented in Taiwan,[8,16] Japan,[15] and Canada,[22] some cases are still not detected in the early ages, resulting in delayed treatment. Therefore, other markers are required to distinguish between infants with BA and prolonged neonatal jaundice in the early stages. D-bil or conjugated bilirubin measurements are potential alternatives.[18–21]

We tested infants aged 3 to 60 days using 4 methods. Although methods 2 (D/T ratio ≥20%) and 3 (D-bil ≥1.0 mg/dL and D/T ratio ≥20%) had higher specificity (81.3% and 90.3%, respectively), 2 patients with BA were missed by those methods, which had a sensitivity of 94.4%. Methods 1 (D-bil ≥1.0 mg/dL) and 4 (D-bil ≥1.0 mg/dL or D/T ratio ≥20%) had the highest sensitivity and NPV (both 100%), although method 1 had greater specificity (77.3% vs 68.3%). These results were also consistent with the ROC curve comparison between D-bil and the D/T ratio (Fig 3), suggesting that D-bil measurement was better for detecting BA in infants aged 3 to 60 days than the D/T ratio, especially for infants aged >30 days. Hodgson et al[23] performed a retrospective cohort analysis of infants aged 10 to 70 days with prolonged neonatal jaundice and found that the T-bil and D-bil levels decreased with age in healthy infants, whereas the D/T ratio was highly variable. In our study, we also found that some healthy infants had D/T ratios ≥20%, especially when T-bil was <5.0 mg/dL. Furthermore, 4 patients with BA had initial D/T ratios <20%. Therefore, consistent with current guidelines, D-bil ≥1.0 mg/dL was found to be better for detecting BA.[4]

For newborns aged <3 days, a D-bil cutoff of ≥0.5 mg/dL was used but had poor specificity and sensitivity. Although D-bil ≥0.5 mg/dL was considered to indicate BA, it may not be the optimal cutoff point for our patient population. Furthermore, the upper limits of normal D-bil in early life may vary among laboratories. In the ROC curve analysis, the sensitivity and specificity were 100% and 14.3%, respectively, using D-bil >0.45 mg/dL as the cutoff point. Harpavat et al[18] demonstrated elevated D-bil levels in patients with BA during the first 24 hours of life (0.98 ± 0.17 mg/dL), with higher mean D-bil levels seen in patients with BA compared with healthy infants (1.4 ± 0.43 vs 0.19 ± 0.075 mg/dL; $P < .0001$) at 24 to 48 hours after birth. The same authors recently published a cross-sectional study describing a 2-stage program conducted in Texas for BA screening.[20] D-Bil or conjugated bilirubin levels were measured within 60 hours of birth, followed by a second test within 2 weeks after birth for those with abnormal findings. The sensitivity and specificity were 100% and 99.9%, respectively. Thus, a second test is important at this age, especially in cases with an abnormal first test or persistent jaundice.

The prevalence of cholestatic liver disease in our study was 7.5% (334 of 4468), which was higher than that in the general population. One reason for this finding was that the enrolled infants had prolonged jaundice or were suspected of having cholestatic liver disease and thus, were not representative of the general population. Second, the most common causes of cholestatic liver disease in our patients were TPN cholestasis, neonatal hepatitis, and

TABLE 3 Various Comparisons in Different Selected Diseases Between Ages 3 and 60 Days

	BA (n = 36)	TPN Cholestasis (n = 76)	Neonatal Hepatitis (n = 62)	Heart Disease With Shock-Related Cholestatic Liver Disease (n = 54)	Infection[a] (n = 32)	Alagille Syndrome[b] (n = 6)	NICCD (n = 5)	P
Testing age, d	29 ± 18.2	26 ± 16.0	36 ± 15.4	20 ± 14.8	28 ± 15.7	36 ± 18.5	41 ± 19.3	<.001[c]
D-bil, mg/dL	4.4 ± 1.5	3.5 ± 2.4	3.7 ± 3.6	2.7 ± 3.3	3.8 ± 2.8	3.9 ± 1.1	2.4 ± 1.4	.163
T-bil, ng/dL	8.8 ± 2.8	7.7 ± 3.9	8.1 ± 5.0	8.7 ± 6.2	9.6 ± 4.2	7.7 ± 1.8	8.9 ± 3.7	.609
D/T ratio, %	53 ± 16.7	48 ± 20.7	44 ± 20.8	35 ± 24.7	43 ± 23.1	52 ± 15.6	31 ± 17.5	.002[d]

Data are presented as mean ± SD. NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency.

[a] Including urinary tract infection and sepsis.

[b] Including paucity of intrahepatic bile duct.

[c] $P < .05$ between neonatal hepatitis and heart disease with shock-related cholestatic liver disease.

[d] $P < .05$ between BA and heart disease with shock-related cholestatic liver disease.

heart disease and shock-related cholestatic liver disease. We did not exclude premature infants, those with complex congenital heart diseases, or patients under intensive care. Therefore, there may have been a selection or referral bias, leading to an underestimation of specificity. It is a challenge to distinguish these conditions from BA because of the complex disease course. Therefore, follow-up tests are necessary in infants with abnormal findings or persistent jaundice.[24]

Our study had several limitations. First, the data were collected in a referral center and may not represent the initial data. Second, patients with BA lost to follow-up were not included in the analysis. Third, our study was not based on a universal screening program for infants aged <60 days, which might have led to a selection bias. As an example, we enrolled some premature infants and patients under intensive care who were at a greater risk for prolonged jaundice.

CONCLUSIONS

D-bil measurement in infants with jaundice is useful for early detection of BA. D-bil ≥1.0 mg/dL alone is better than the D/T ratio in infants aged 3 to 60 days and has an optimal sensitivity and NPV. For newborns aged <3 days, D-bil >0.45 mg/dL is the acceptable cutoff point, and a second test is essential. We suggest that D-bil levels be measured for all infants with prolonged neonatal jaundice or abnormal stool color. Prospective studies in larger samples are required to confirm the utility and cost effectiveness of this method.

ABBREVIATIONS

BA: biliary atresia
CI: confidence interval
D-bil: direct bilirubin
D/T: direct to total bilirubin
ICD-9: *International Classification of Diseases, Ninth Revision*
ICD-10: *International Classification of Diseases, Tenth Revision*
NPV: negative predictive value
PPV: positive predictive value
ROC: receiver operating characteristic
T-bil: total bilirubin
TPN: total parenteral nutrition

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