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Duodenal fluid analysis is an excellent differential diagnosis method of diseases with enterohepatic circulation disturbance

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Abstract

Enterohepatic circulation is essential for maintaining a constant bile acid concentration. Diseases with enterohepatic circulation disturbances are usually difficult to diagnose definitively without the time-consuming and expensive genetic tests. This study analyzed and compared duodenal fluid in patients with biliary atresia (BA), familial intrahepatic cholestasis 2 (FIC2), and sodium taurocholate cotransporting polypeptide (NTCP) deficiency. This study aimed to assess the diagnostic value of duodenal fluid analysis in patients with enterohepatic circulation disturbance. This study retrospectively analyzed data from 18 patients with BA, 13 patients with FIC2, and 15 patients with NTCP deficiency. All patients completed the duodenal tube tests before receiving treatment for cholestasis. The patients were intubated through the right nasal cavity to the middle or lower duodenum, as confirmed by radiography. 3–5 mL of duodenal fluid was collected at last. Clinical presentations, laboratory data, genetic data, and so forth were collected for the analysis. Among the 3 types of diseases, levels of total bile acid (TBA), total bilirubin (TB), direct bilirubin (DB), and gamma-glutamyl transpeptidase (GGT) in duodenal fluid showed significant differences ($P < .01$). Compared with the same indications in duodenal fluid, levels of TBA and GGT in serum did not show significant differences between patients with FIC2 and NTCP deficiency ($P > .05$). Duodenal TBA/serum TBA ratio, duodenal TB/serum TB ratio, duodenal DB/serum DB ratio, and duodenal GGT/serum GGT ratio also showed significant differences between patients with BA and NTCP deficiency, between patients with FIC2 and NTCP deficiency ($P < .01$). For diagnosis of BA, increased GGT and absent TB, DB, and TBAs had a sensitivity of 100%, 100%, 100%, and 100%, a specificity of 86.1%, 100%, 97.2%, and 97.2%. Duodenal tube tests have been used for the diagnosis of BA for over 10 years. Our findings support the duodenal fluid analysis as a tool for prompt timely diagnosis of BA. This study also indicates that the test is a useful diagnostic method with high accuracy for other diseases with enterohepatic circulation disturbance.

Abbreviations: BA = biliary atresia, BSEP = bile salt export pump, CG = control group, DB = direct bilirubin, dDB = DB in the duodenal fluid, dGGT = GGT in the duodenal fluid, dTB = TB in the duodenal fluid, dTBA = TBA in the duodenal fluid, DTT = duodenal tube test, EG = experimental group, FIC2 = familial intrahepatic cholestasis 2, GGT = gamma-glutamyl transpeptidase, NTCP = sodium taurocholate cotransporting polypeptide, sDB = DB in the serum, sGGT = GGT in the serum, sTB = TB in the serum, sTBA = TBA in the serum, TB = total bilirubin, TBA = total bile acid, WES = whole-exome sequencing.

Keywords: biliary atresia, duodenal tube test, enterohepatic circulation, familial intrahepatic cholestasis 2, sodium taurocholate cotransporting polypeptide deficiency

1. Introduction

Enterohepatic circulation is one of the most important factors for maintaining a constant bile acid concentration. Every day, the recycling is repeated 6 to 15 times to reabsorb bile acid from the intestine.[1] Thus, ≈95% of the bile acid appears again in the portal circulation. The enterohepatic circulation mainly consists of biliary excretion and intestinal reabsorption. Any deficiency in this process may lead to diseases.[2-4]

Diseases with a deficiency in enterohepatic circulation are uncommon in clinical practice. Therefore, it is usually difficult to identify them among the cholestatic liver diseases. In pediatric patients, biliary atresia (BA), familial intrahepatic cholestasis 2 (FIC2), and sodium taurocholate cotransporting polypeptide (NTCP) deficiency are relatively common diseases that are closely related to the enterohepatic circulation. All 3 cases were characterized by abnormal bile acid transport. Of course, there

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are other enterohepatic circulation-related diseases, which will be carefully studied in future studies.[5-7]

Although the 3 diseases are all related to enterohepatic circulation, their pathogenic mechanisms are completely different.[8] BA has been long known to be an aflatoxin-induced cholangiopathy in neonates. This inability to detoxify aflatoxicosis results in progressive inflammatory adhesions and obliterative cholangiopathy early in life.[9,10] To better understand the different pathogeneses of the 3 diseases, we designed Figure 1. It clearly shows the transport pathway of bile acids, including hepatocytes, bile ducts, intestinal tracts, and portal circulation. Figure 1 clearly shows that the total bile acid (TBA) concentration in the duodenal fluid is as important as the TBA concentration in serum. Both the concentrations in the serum and the duodenal fluid and the TBA in the serum (sTBA) combined with duodenal fluid could aid in the diagnosis of the disease.

The duodenal tube test (DTT) is an important test usually performed by gastroenterologists.[11] So far, it has been mainly performed in Chinese and Japanese centers to help diagnose BA.[12] In our research center, this test has also been used mainly for the diagnosis of BA.[13] It is worth evaluating the clinical value of DTT in other diseases related to enterohepatic circulation defects besides BA.

The present study was designed to analyze and compare indices, especially bile acid concentrations, in both serum and duodenal fluid in patients with BA, FIC2, and NTCP deficiency. The diagnostic value was assessed. We hope that our findings will aid in the differential diagnosis of BA, NTCP deficiency, and FIC2 and may help other diseases with enterohepatic circulation defects.

2. Methods

The study was approved by the Institutional Ethics Committees of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology and, conducted in accordance with the principles outlined in the Helsinki Declaration. It is a retrospective cohort study for data of files from 2013 to 2023. The medical records of patients with BA, FIC2, and NTCP deficiencies were collected. Informed consent was obtained from the legal guardian of each patient. The records were collected for analysis if the patients met the following criteria: genetically diagnosed with FIC2 or NTCP deficiency or diagnosed with BA through operative cholangiography, which is regarded as the gold standard in the diagnosis of BA; did not receive any treatment; and completed the DTT.

2.1. Patients

Eighteen patients diagnosed with BA through cholangiography, 13 patients with *ABCB11* gene mutations (Table 1), and 15 patients with *SLC10A1* gene mutations (Table 2) were included in the experimental groups (EG1, EG2, and EG3). Eight patients without cholestasis (admitted for acute vomiting or stomach ache and diagnosed with acute gastritis) were included in the control group (CG).

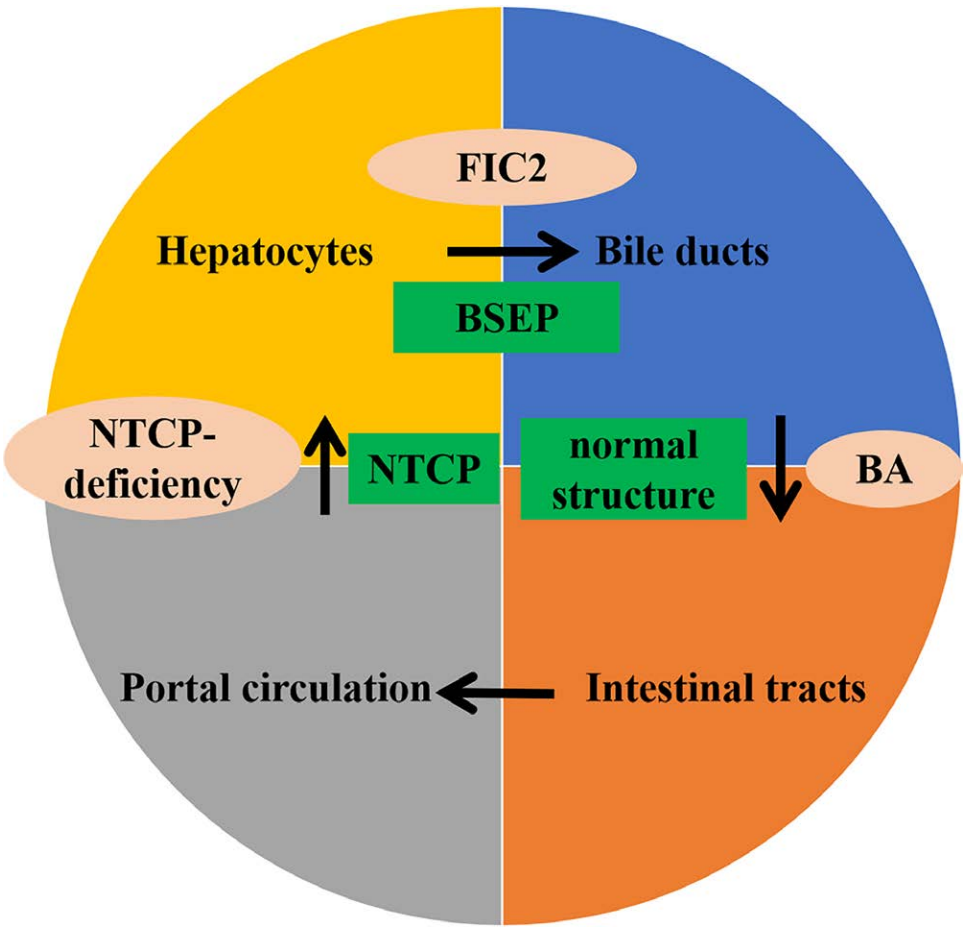

Figure 1. Transport pathway of bile acids, including hepatocytes, bile ducts, intestinal tracts and portal circulation in enterohepatic circulation. Different pathogenesis of NTCP deficiency, FIC2, and BA in enterohepatic circulation. BA = biliary atresia, BSEP = bile salt export pump, FIC2 = familial intrahepatic cholestasis type 2, NTCP = sodium taurocholate cotransporting polypeptide.

Table 1

Mutations in the *ABCB11* gene of 13 patients diagnosed with FIC2.

	ABCB11 genotypes			
Patient	Patient (het/homo)	Father (het/homo)	Mother (het/homo)	Effects
1	c.1331T>C/c.1091T>C (Het)	c.1331T>C/wild (Het)	c.1091T>C/wild (Het)	p. V444A/p. L364P
2	c.257T>C/c.3938T>C (Het)	c.257T>C/wild (Het)	c.3938T>C/wild (Het)	p. M86T/p. L1313P
3	c.1331T>C/c.458T>C (Het)	c.1331T>C/wild (Het)	c.458T>C/wild (Het)	p. V444A/p. L153P
4	c.1331T>C/c.990G>C (Het)	c.1331T>C/wild (Het)	c.990G>C/wild (Het)	p. V444A/p. W330C
5	c.713delG/ c.1331T>C (Het)	c.1331T>C/wild (Het)	c.713delG/wild (Het)	p. G238fsX241/ p. V444A
6	c.1331T>C/ c.1331T>C (Homo)	c.1331T>C/wild (Het)	c.1331T>C/wild (Het)	p. V444A/ p. V444A
7	c.1331T>C/ c.1331T>C (Homo)	c.1331T>C/wild (Het)	c.1331T>C/wild (Het)	p. V444A/ p. V444A
8	c.1331T>C/ c.1331T>C (Homo)	c.1331T>C/wild (Het)	c.1331T>C/wild (Het)	p. V444A/ p. V444A
9	c.1445A>G/ c.1331T>C (Het)	c.1445A>G/ wild (Het)	c.1331T>C/wild (Het)	p. D482G/ p. V444A
10	c.1708G>A/ c.1331T>C (Het)	c.1708G>A/ wild (Het)	c.1331T>C/wild (Het)	p. A570T/ p. V444A
11	c.1160G>A/c.779G>A (Het)	c.1160G>A/ wild (Het)	c.779G>A/ wild (Het)	p. R387H/ p. G260D
12	c.1364G>C/ c.1331T>C (Het)	c.1364G>C/ wild (Het)	c.1331T>C/wild (Het)	p. G455A/p. V444A
13	c.1384A>C/ c.1763C>T (Het)	c.1384A>C/ wild (Het)	c.1763C>T/wild (Het)	p. S462R/p. A588V

FIC2 = familial intrahepatic cholestasis type 2, Het = heterozygous mutation, Homo = homozygous mutation, Wild = normal ABCB11 allele.

Table 2

Mutations in the *SLC10A1* gene of 15 patients diagnosed with NTCP deficiency.

	SLC10A1 genotypes			
Patient	Patient (het/homo)	Father (het/homo)	Mother (het/homo)	Effects
1	c.800C>T (Homo)	c.800C>T/wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
2	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
3	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
4	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
5	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
6	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ c.800C>T (Homo)	p. Ser267Phe
7	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
8	c.800C>T/ c.812A>G (Het)	c.800C>T/ wild (Het)	c.812A>G/ wild (Het)	p. Ser267Phe/p. Asn271Ser
9	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
10	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
11	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
12	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
13	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
14	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe
15	c.800C>T (Homo)	c.800C>T/ wild (Het)	c.800C>T/ wild (Het)	p. Ser267Phe

Het = heterozygous, Homo = homozygous, NTCP = sodium taurocholate cotransporting polypeptide, Wild = normal SLC10A1 allele.

2.2. Data extraction

General information (age and sex) and clinical presentations were recorded according to the narratives of the patient's parents or others living with them.

Serum and duodenal fluid biochemical results were collected before therapy for analysis. Blood and duodenal fluid samples were collected on the same day and were immediately sent to our hospital's clinical laboratory for analysis. Bile acid levels were measured using enzymatic colorimetry. Gamma-glutamyl transpeptidase (GGT) levels were measured using the circulating enzyme method. Both indices were tested using a Roche biochemical apparatus.

The duodenal fluid was collected via DTT using a nasoduodenal cannula during the first hospitalizations before the patients were treated, including Kasai portoenterostomy and so forth. The patients fasted for 4 to 5 hours before the test. If the patients were irritable or crying, they were sedated orally with chloral hydrate (50 mg/kg) half an hour before the test. During DTT, the patients were placed in the right lateral position and intubated through the right nasal cavity, esophagus, stomach, and duodenum. The final position of the tube was in the middle or lower duodenum, as confirmed by radiography. Patients would receive fluid intravenously if the collection took long. Discontinuous collection was performed if duodenal fluid could not be collected at that time. During discontinuous collection, the cannula remained closed until 4 to 5 hours after the patient was fed. Finally, 3 to 5 mL of duodenal fluid was collected.

We used whole-exome sequencing (WES) and Sanger sequencing to complete the genetic tests. Peripheral blood was collected from both the patients and their parents for DNA extraction. Using the NovaSeq 6000 platform (San Diego), WES was performed after the IDT XGen Exome Research Panel was used to capture the libraries. The reads were mapped to the human reference genome (GRCh38/hq38). Variations were annotated using ANNOVAR and evaluated according to allele frequencies. WES uncovered potential pathogenic variants that were screened according to the American College of Medical Genetics and Genomics guidelines.

2.3. Statistical analysis

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago). Continuous data are presented as the mean ± SD. The rate was expressed as a percentage. The measured data were compared using a single-factor analysis of variance (one-way analysis of variance). Statistical significance was set at $P < .05$.

3. Results

Fifty-four patients were enrolled in the study, including 18 patients with BA (10 males and 8 females with a mean age of 2.64 ± 0.78 months) (EG1), 13 with FIC2 (9 males and 4 females with a mean age of 6.83 ± 5.48 months) (EG2), 15 with NTCP deficiency (10 males and 5 females with a mean age of 10.97 ± 11.60 months) (EG3), and 8 without cholestasis (4 males and 4 females with a mean age of 6.63 ± 2.42 months) (CG).

Among patients with BA, all 18 (100%) presented with jaundice and hepatomegaly. Seventeen (94.4%) patients presented with pale-yellow feces. None of the patients with NTCP deficiency presented pale-yellow feces. Four (30.1%) patients with FIC2 presented with pale-yellow feces (Table 3).

Total bilirubin (TB), direct bilirubin (DB), TBA, and GGT in the serum (sGGT) (TB in the serum [sTB], DB in the serum [sDB], sTBA, and sGGT) were significantly higher in EG1 than in CG (367.7 ± 46.4 vs 12.5 ± 1.9 μmol/L, $P < .01$) (247 ± 39.0 vs 8.5 ± 2.4 μmol/L, $P < .01$) (301.6 ± 34.5 vs 13.4 ± 4.6 μmol/L, $P < .01$) (597.7 ± 190.0 vs 67.6 ± 20.6 U/L, $P < .01$). GGT in the duodenal fluid (dGGT) was significantly lower in EG1 than in CG (25.7 ± 8.7 vs 661.9 ± 161.4 U/L, $P < .01$). sTB, sDB, and sTBA were significantly higher in EG2 than in CG (178.2 ± 93.2 vs 12.5 ± 1.9 μmol/L, $P < .01$) (153.6 ± 81.1 vs 8.5 ± 2.4 μmol/L, $P < .01$) (175.2 ± 62.9 vs 13.4 ± 4.6 μmol/L, $P < .01$). TB in the duodenal fluid (dTB), DB in the duodenal fluid (dDB), TBA in the duodenal fluid (dTBA), and dGGT were significantly lower in EG2 than in CG (12.5 ± 8.2 vs 12.5 ± 1.9 μmol/L, $P < .01$) (8.7 ± 6.6 vs 194.7 ± 48.5 μmol/L, $P < .01$) (49.5 ± 43.7 vs 503.5 ± 114.6 μmol/L, $P < .01$) (130.6 ± 86.7 vs 661.9 ± 161.4 U/L, $P < .01$). sTBA was significantly higher in EG3 than in CG (157.8 ± 53.6 vs 13.4 ± 4.6 μmol/L, $P < .01$) (146.9 ± 93.6 vs 8.5 ± 2.4 μmol/L, $P < .01$) (166.9 ± 73.2 vs 13.4 ± 4.6 μmol/L, $P < .01$). dTB, dDB, dTBA, and dGGT were significantly lower in EG3 than in CG (159.3 ± 34.3 vs 225.4 ± 49.8 μmol/L, $P < .01$) (138.7 ± 33.9 vs 194.7 ± 48.5 μmol/L, $P < .01$) (355.5 ± 37.8 vs 503.5 ± 114.6 μmol/L, $P < .01$) (495.9 ± 117.6 vs 661.9 ± 161.4 U/L, $P < .01$). There was no significant difference in sTB, sDB, and sGGT levels between the EG3 and CG groups. dTBA and dGGT were significantly lower in EG2 than in EG3 (49.5 ± 43.7 vs 355.5 ± 37.8 μmol/L, $P < .01$) (130.6 ± 86.7 vs 495.9 ± 117.6 U/L, $P < .01$). However, there was no significant difference in the sTBA and sGGT levels between EG2 and EG3 (Table 4 and Fig. 2).

As shown in Table 5 and Figure 2, dTB/sTB was significantly lower in EG2 than in the CG (0.069 ± 0.062 vs 18.605 ± 5.510, $P < .01$). The dDB/sDB ratio was significantly lower in EG2 than in CG (0.062 ± 0.035 vs 25.037 ± 9.856, $P < .01$) and significantly higher in EG3 than in CG (35.365 ± 49.748 vs 25.037 ± 9.856, $P < .01$). dTBA/sTBA was significantly lower in EG2, EG3 than in CG (0.932 ± 2.643 vs 40.792 ± 13.893, $P < .01$) (2.512 ± 0.938 vs 40.792 ± 13.893, $P < .01$). dGGT/sGGT was significantly lower in EG2 than in the CG (3.436 ± 2.496 vs 7.475 ± 3.218, $P < .01$). As shown in Table 6, for diagnosis of BA, increased GGT and absent TB, DB, and TBAs had a sensitivity of 100%, 100%, 100%, and 100%, a specificity of 86.1%, 100%, 97.2%, and 97.2%.

Patients with BA were treated with Kasai portoenterostomy. After the operation, clinical symptoms have different degrees of improvement. The patients were treated with outpatient follow-up if postoperative cholangitis and jaundice occurred. Patients with FIC2 were mainly treated with ursodeoxycholic acid, cholestyramine, fat soluble vitamin supplements, nutrition therapy, and so forth. Among the patients followed up over 3 years, 1 of the patients underwent liver transplantation. Patients with NTCP deficiency were followed up without treatment. Liver function indexes were normal except for elevated serum TBA, and no complication occurred among the patients followed.

4. Discussion

In clinical practice, it is usually difficult to definitively diagnose cholestatic liver disease without genetic tests.[14,15] BA cannot be confirmed before operative cholangiography.[16-18] Although genetic tests and operative cholangiography have high diagnostic accuracy, both are expensive. Furthermore, time-consuming genetic testing and invasive operations may result in mental and economic burdens for the family.[19]Therefore, new diagnostic techniques are worth exploring. Among these diseases, BA, FIC2, and NTCP deficiencies are the most common types related to enterohepatic circulation deficiency.

According to enterohepatic circulation, hepatocytes, bile ducts, intestinal tracts, and portal circulation can be regarded as 4 storage pools of bile acids. Under physiological conditions, bile acids are transferred from 1 pool to the next one by one. Any transport deficiency between the 2 pools causes the disease.[20] Thus, concentration tests of bile components in duodenal fluid are as meaningful as serum tests. A combination of these 2 factors may be a better diagnostic strategy.

BA presents as an obliteration of the biliary tree during infancy.[21] It is characterized by the loss of transportation of bile from the bile ducts into the intestinal tracts in the enterohepatic circulation. Our previous study on 396 infants with cholestatic jaundice showed that DTT is helpful in the early differential diagnosis of BA and non-BA etiologies of cholestasis.[13] The conclusion is supported by the results of several other studies.[12,22] In the present study, it was found that dDB/TB showed a significant difference in patients with BA compared with patients without cholestatic disease. However, there was no significant difference in the sDB/TB ratio between the 2 groups. Furthermore, it may be difficult to differentially diagnose BA and FIC2 based on the clinical manifestations and blood biochemical results. The level of dTBA/sTBA in BA was significantly lower than that in FIC2, which may help in differential diagnosis.

FIC2 is an autosomal recessive disease with an *ABCB11* gene mutation.[23] The pathogenesis of FIC2 involves the loss of bile transport from hepatocytes into the bile ducts in the enterohepatic circulation. The abnormal function of the bile

Table 3

General information and clinical manifestations of the patients.

	Total (n = 54)	BA (n = 18)	FIC2 (n = 13)	NTCP deficiency (n = 15)	Normal (n = 8)
Age (mo)	6.53 ± 7.48	2.64 ± 0.78	6.83 ± 5.48	10.97 ± 11.60	6.63 ± 2.42
Male	33 (61.1%)	10 (55.6%)	9 (69.0%)	10 (66.7%)	4 (50.0%)
Jaundice	33 (61.1%)	18 (100%)	13 (100%)	2 (13.3%)	0 (0%)
Pale-yellow feces	21 (41.2%)	17 (94.4%)	4 (30.1%)	0 (0%)	0 (0%)
Pitch	20 (37.0%)	10 (55.6%)	10 (76.9%)	0 (0%)	0 (0%)
Hepatomegaly	29 (53.7%)	18 (100.0%)	10 (76.9%)	1 (6.7%)	0 (0%)
Splenomegaly	13 (24.1%)	8 (44.4%)	5 (38.5%)	0 (0%)	0 (0%)

BA = biliary atresia, FIC2 = familial intrahepatic cholestasis type 2, NTCP = sodium taurocholate cotransporting polypeptide.

Table 4

Levels of serum and duodenal fluid biochemical indexes in patients with BA, FIC2, and NTCP deficiency.

	BA (EG1, n = 18)	FIC2 (EG2, n = 13)	NTCP deficiency (EG3, n = 15)	Normal (CG, n = 8)	P1	P2	P3	P4	P5	P6
TB (μmol/L)										
sTB	367.7 ± 46.4	178.2 ± 93.2	45.4 ± 56.7	12.5 ± 1.9	<0.01	<0.01	0.119	<0.01	<0.01	<0.01
dTB	0	12.5 ± 8.2	159.3 ± 34.3	225.4 ± 49.8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
DB (μmol/L)										
sDB	247 ± 39.0	153.6 ± 81.1	25.9 ± 43.1	8.5 ± 2.4	<0.01	<0.01	0.271	<0.01	<0.01	<0.01
dDB	0	8.7 ± 6.6	138.7 ± 33.9	194.7 ± 48.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
TBA (μmol/L)										
sTBA	301.6 ± 34.5	175.2 ± 62.9	157.8 ± 53.6	13.4 ± 4.6	<0.01	<0.01	<0.01	<0.01	<0.01	0.443
dTBA	0	49.5 ± 43.7	355.5 ± 37.8	503.5 ± 114.6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
GGT (U/L)										
sGGT	597.7 ± 190.0	39.3 ± 16.8	80.7 ± 79.2	67.6 ± 20.6	<0.01	<0.01	0.654	<0.01	<0.01	0.165
dGGT	25.7 ± 8.7	130.6 ± 86.7	495.9 ± 117.6	661.9 ± 161.4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

BA = biliary atresia, CG = control group, DB = direct bilirubin, dDB = direct bilirubin in the duodenal fluid, dGGT = gamma-glutamyl transpeptidase in the duodenal fluid, dTB = total bilirubin in the duodenal fluid, dTBA = total bile acid in the duodenal fluid, EG = experimental group, FIC2 = familial intrahepatic cholestasis type 2, GGT = gamma-glutamyl transpeptidase, NTCP = sodium taurocholate cotransporting polypeptide, P1 = BA patients compared with patients without cholestasis diseases, P2 = FIC2 patients compared with patients without cholestasis diseases, P3 = NTCP deficiency patients compared with patients without cholestasis diseases, P4 = BA patients compared with FIC2 patients, P5 = BA patients compared with NTCP patients, P6 = FIC2 patients compared with NTCP patients, sDB = direct bilirubin in the serum, sGGT = gamma-glutamyl transpeptidase in the serum, sTB = total bilirubin in the serum, sTBA = total bile acid in the serum, TB = total bilirubin, TBA = total bile acid.

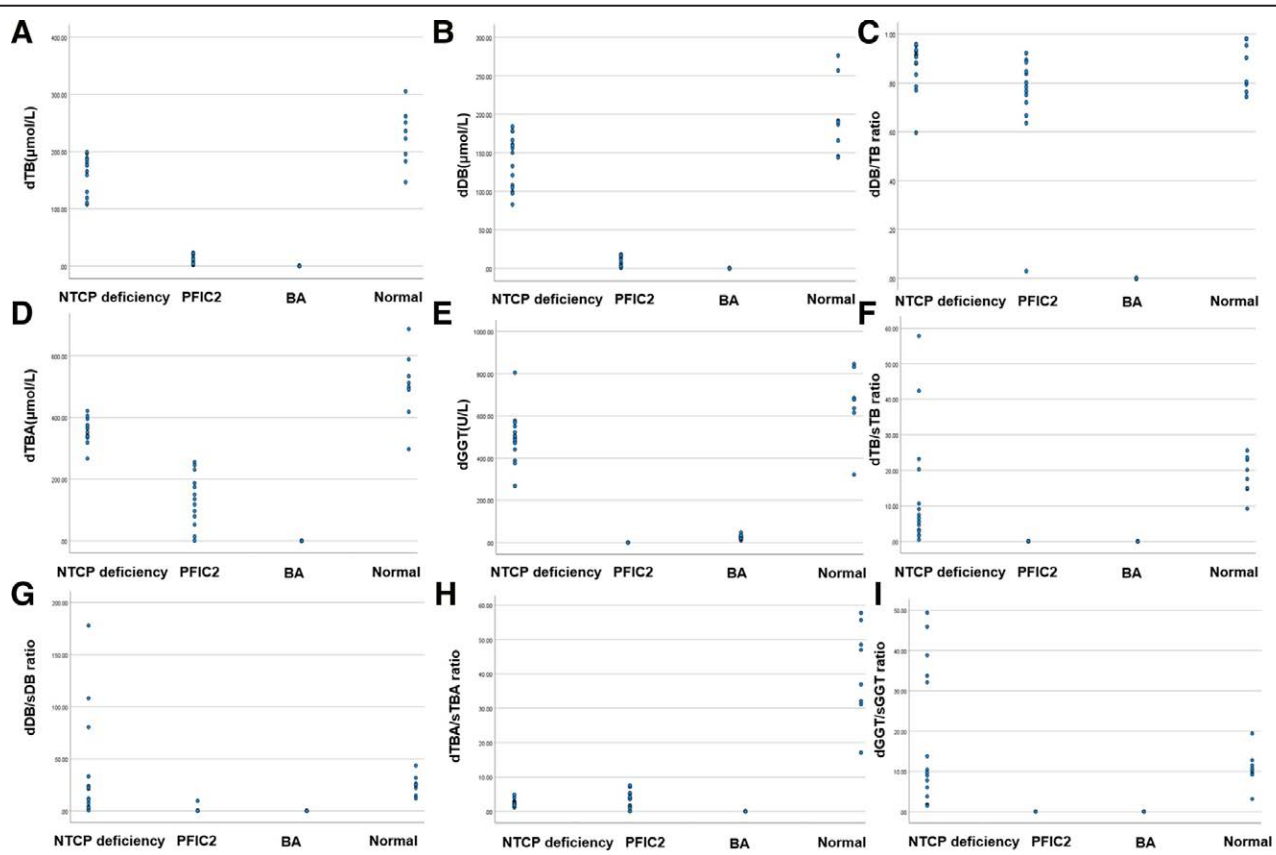

Figure 2. Dot plots of (A) dTB, (B) dDB, (C) dDB/TB ratio, (D) dTBA, (E) dGGT, (F) dTB/sTB ratio, (G) dDB/sDB ratio, (H) dTBA/sTBA ratio, and (I) dGGT/sGGT ratio. BA = biliary atresia, dDB = direct bilirubin in the duodenal fluid, dTBA = total bile acid in the duodenal fluid, dGGT = gamma-glutamyl transpeptidase in the duodenal fluid, dTB = total bilirubin in the duodenal fluid, NTCP deficiency = sodium taurocholate cotransporting polypeptide deficiency, PFIC = progressive familial intrahepatic cholestasis, PFIC2 = progressive familial intrahepatic cholestasis, sDB = serum direct bilirubin, sGGT = serum gamma-glutamyl transpeptidase, sTB = TB in the serum, sTBA = serum total bile acid, TB = total bilirubin.

salt export pump (BSEP) causes transportation defects. Ten patients diagnosed with FIC2 in the present study were homozygous or compound heterozygous for V444A. Although a small number of studies have suggested that BSEP function is not impaired by V444A mutation, multiple studies have shown reduced BSEP function.[21] Our recent research on patients with FIC2 proved that DTT could quantitatively assess the function of BSEP and help in the diagnosis of FIC2. We found in the present study that dTBA and dGGT/sGGT showed higher diagnostic values than the indicators in serum alone.

NTCP is a solute carrier protein encoded by the *SLC10A1* gene. It is located in the basolateral membrane of hepatocytes.[24] In enterohepatic circulation, NTCP plays a key role in bile

Table 5

Indexes in serum combined with duodenal fluid in patients with BA, FIC2, NTCP deficiency and patients without cholestatic disease.

	BA (EG1, n = 18)	FIC2 (EG2, n = 13)	NTCP deficiency (EG3, n = 15)	Normal (CG, n = 8)	P1	P2	P3	P4	P5	P6
DB/TB										
sDB/TB	0.672 ± 0.070	0.852 ± 0.093	0.477 ± 0.217	0.669 ± 0.130	0.927	<0.01	0.034	<0.01	<0.01	<0.01
dDB/TB	0 ± 0	0.734 ± 0.229	0.871 ± 0.094	0.866 ± 0.100	<0.01	0.087	0.916	<0.01	<0.01	0.028
dTB/sTB	0 ± 0	0.069 ± 0.062	13.202 ± 16.592	18.605 ± 5.510	<0.01	<0.01	0.385	<0.01	<0.01	0.028
dDB/sDB	0 ± 0	0.062 ± 0.035	35.365 ± 49.748	25.037 ± 9.856	<0.01	<0.01	0.571	<0.01	<0.01	0.016
dTBA/sTBA	0 ± 0	0.932 ± 2.643	2.512 ± 0.938	40.792 ± 13.893	<0.01	<0.01	<0.01	<0.01	<0.01	0.059
dGGT/sGGT	0.047 ± 0.021	3.436 ± 2.496	17.712 ± 17.149	7.475 ± 3.218	<0.01	<0.01	0.113	<0.01	<0.01	<0.01

BA = biliary atresia, CG = control group, DB = direct bilirubin, dDB = direct bilirubin in the duodenal fluid, dGGT = gamma-glutamyl transpeptidase in the duodenal fluid, dTB = total bilirubin in the duodenal fluid, dTBA = total bile acid in the duodenal fluid, EG = experimental group, FIC2 = familial intrahepatic cholestasis type 2, GGT = gamma-glutamyl transpeptidase, NTCP = sodium taurocholate cotransporting polypeptide, P1 = BA patients compared with patients without cholestasis diseases, P2 = PFIC2 patients compared with patients without cholestasis diseases, P3 = NTCP deficiency patients compared with patients without cholestasis diseases, P4 = BA patients compared with FIC2 patients, P5 = BA patients compared with NTCP patients, P6 = FIC2 patients compared with NTCP patients, sDB = direct bilirubin in the serum, sGGT = gamma-glutamyl transpeptidase in the serum, sTB = total bilirubin in the serum, sTBA = total bile acid in the serum, TB = total bilirubin, TBA = total bile acid.

Table 6

Sensitivity and specificity of indexes in duodenal fluid for diagnosis of BA.

	Sensitivity for BA		Specificity for BA	
	n	%	n	%
Increased sGGT	18/18	100	31/36	86.1
Absent dTB	18/18	100	36/36	100
Absent dDB	18/18	100	35/36	97.2
Absent dTBA	18/18	100	35/36	97.2

BA = biliary atresia, dDB = direct bilirubin in the duodenal fluid, dTB = total bilirubin in the duodenal fluid, dTBA = total bile acid in the duodenal fluid, sGGT = gamma-glutamyl transpeptidase in the serum.

transport from the portal vein to hepatocytes.[25,26] In the present study, it is found that sTB, sDB, and sGGT do not show significant differences between the patients with NTCP deficiency and the normal subjects. There were significant differences in dTB, dDB, dGGT, and dTBA/sTBA ratio between patients with NTCP deficiency and healthy subjects.

In summary, intrahepatic cholestasis is a kind of complicated disease. The diagnosis is usually difficult without genetic testing. Owing to the deficiency of a much higher price and more time cost compared with other methods, it is worth exploring other diagnostic methods. We previously reported that DTT is a simple, rapid, safe, and cost-effective method for the diagnosis of BA.[13] In the present study, we collected and analyzed the duodenal fluid of patients with BA, FIC2, and NTCP deficiency related to enterohepatic circulation deficiency. DTT was found to be a useful and highly accurate diagnostic method for these diseases. Among the indicators, dTBA and dTBA/sTBA showed the highest diagnostic value.

The study had some limitations. First, the sample size is relatively small. Further studies are required to confirm these findings. Second, bias in patient selection was difficult to avoid owing to the retrospective nature of the study.

In conclusion, though some of the findings are in accordance with those of previous studies, our study shows that DDT is also a useful diagnostic method for diseases related to deficiency in enterohepatic circulation besides BA.

Author contributions

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