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Original Article

The role of inborn errors of metabolism in the etiology of neonatal cholestasis: A single center experience

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ABSTRACT

Objective: The evaluation of patients with neonatal cholestasis is difficult due to the variety of cholestatic syndromes and non-specific clinical findings. It is important to recognize treatable diseases promptly. The aim of this study is to draw attention to suspicious markers in order to diagnose treatable metabolic diseases.

Method: The presented retrospective study included patients with cholestasis in the first three months of life. The study was conducted between 2018 and 2021 at Diyarbakır Children's Hospital, Türkiye.

Results: 253 patients presenting with neonatal cholestasis were retrospectively evaluated. 174 patients (68.77%) were examined for intrahepatic cholestasis. 16.6% of the patients were diagnosed with an infection, 13.43% with TPN-related cholestasis, 8.3% with IEM, 7.11% with cystic fibrosis, 4.74% with endocrinopathy, 4.34% patients with Alpha-1 antitrypsin deficiency, 2.76% with idiopathic neonatal hepatitis, 1.97% with genetic syndrome, 1.58% with PFIC, and 0.79% patients with Alagille syndrome. IEM-related patients (21) were diagnosed with tyrosinemia type 1, galactosemia, Niemann-Pick type A, glycogen storage disease type 3, peroxisomal disorders, fatty acid oxidation defects, mitochondrial DNA depletion syndrome, citrine deficiency, Niemann-Pick Type C and bile acid synthesis defect. Plasma tyrosine and methionine levels were high in patients with not only tyrosinemia type 1, but also galactosemia and citrine deficiency. Therapeutic plasma exchange was performed in two patients with fatty acid oxidation disorders.

Conclusion: Neonatal cholestasis poses a diagnostic challenge for clinicians. Delayed referral to a specialist for treatable metabolic diseases may increase mortality and morbidity. IEMs are observed more frequently in the etiologies of neonatal cholestasis in Türkiye due to high parental consanguinity and inadequate newborn screening programs. Treatable disorders should be considered early, as therapeutic interventiosn can be lifesaving. It also helps in genetic counseling, prenatal diagnosis for future pregnancies.

Keywords: Neonatal cholestasis, intrahepatic cholestasis, galactosemia, hereditary tyrosinemia

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INTRODUCTION

Neonatal cholestasis (NC) is a condition characterized by jaundice and conjugated hyperbilirubinemia that begins in the first months of life and affects approximately 1 in 2500 live births.[1] Etiologic categories include both extrahepatic and intrahepatic disorders, such as extrahepatic biliary atresia, idiopathic neonatal hepatitis, stones, plugs or sclerosis of the biliary tract, infections, total parenteral nutrition (TPN) related, endocrinopathies, chromosomal abnormalities, vascular abnormalities, toxin and drug exposures, hypoxia/ischemia, and inborn errors of metabolism.[2] Clinical and laboratory findings of many diseases with neonatal cholestasis are similar to each other. It is important to distinguish between intrahepatic and extrahepatic causes and to recognize treatable diseases promptly. It is extremely important to take a detailed history and perform a complete physical and neurological examination to formulate possible differential diagnoses. This approach is lifesaving and it should not be disregarded that inborn errors of metabolism (IEM) are underdiagnosed.[3] The most common metabolic etiologies of neonatal cholestasis are tyrosinemia type 1, galactosemia, Niemann Pick Type A, B, C, fatty acid oxidation disorders, urea cycle disorders, bile acid synthesis defects, progressive familial intrahepatic cholestasis (PFIC) I-III, peroxisomal disorders, and mitochondrial DNA depletion syndromes.[3,4] Diagnosis can be guided by a detailed history as well as pathological changes of blood ammonia, glucose, lactate, ketone bodies, and pH. Diagnostic laboratory evaluation of neonatal cholestasis should be comprehensive and initiated early. In some diseases, such as hereditary tyrosinemia type 1, galactosemia, and citrin deficiency, the outcome is excellent with early diagnosis and treatment.[5] Even in the absence of effective treatment, infants with progressive liver disease benefit from medical treatment and optimal nutritional support for complications of cholestasis and possibly cirrhosis. The aim of this study is to evaluate and understand the underlying metabolic causes and consequences of patients with neonatal cholestasis. It is also intended to draw attention to suspicious markers in order not to underdiagnose treatable metabolic diseases.

Here, we report data from patients presenting with neonatal cholestasis (n=253), in which we specifically define the metabolic etiology (n=21), providing a clinically focused overview of the differential diagnosis.

MATERIAL AND METHODS

The present retrospective, cross-sectional, single-center study included patients who presented with direct hyperbilirubinemia with onset in the first three months of life. The study was conducted between 2018 and 2021 at the Pediatric Nutrition and Metabolism and Pediatric Gastroenterology and Hepatology Departments, Diyarbakır Children's Hospital, Türkiye. All samples were analyzed by the same laboratory using the same technique. Plasma amino acid levels were determined by the LC-MS/MS kit (Shimadzu LCMS-8040 Liquid Chromatography Mass Spectrometer, ImmuChrom GmbH kit). Urinary organic acid analysis was semi-quantitative, by modifying the method described by Christou et al.[6] Patients with missing data were excluded. Informed consent was obtained from all individuals. The study was performed in accordance with the Declaration of Helsinki and was approved by the Local Ethics Committee of the Diyarbakir Gazi Yaşargil Research and Training Hospital (Date: 02.07.2021 / No: 819).

Statistical analysis

Statistical analyses of the data were performed using the SPSS software package for Windows software package (ver.18.0; SPSS Inc., Chicago, IL, USA). As descriptive statistics, numbers, and percentages for categorical variables, mean ± standard deviation or median (minimum-maximum) were used for numerical variables. The distribution of data was evaluated using the Shapiro-Wilk test. For numerical comparisons, the Student's t-test or Mann-Whitney U test was used to assess differences between two groups according to the normal distribution of the measured parameters.

RESULTS

253 patients (131/122, M/F) were retrospectively reviewed. 79 patients (31.22%) were referred to another center with the suspected diagnosis of extrahepatic cholestasis after the initial evaluation. 174 patients (68.77%) were examined for intrahepatic cholestasis. 135 (72/63, F/M) patients from 130 different families were diagnosed with non-IEM intrahepatic neonatal cholestasis (IHNC) etiologies. 18 (7.11%) patients were undiagnosed. 42 (16.6%) patients were diagnosed with an infection, 34 (13.43%) patients with TPN-related cholestasis, 21 (8.3%) patients with IEM, 18 (7.11%) patients with cystic fibrosis, 12 (4.74%) patients with endocrinopathy, 11 (4.34%) patients with alpha-1 antitrypsin deficiency, seven (2.76%) patients with idiopathic neonatal hepatitis, five (1.97%) patients with genetic syndrome, four (1.58%) patients with PFIC and two (0.79%) patients with Alagille syndrome in 156 patients. All patients are listed in Figure 1 by etiology. 21 (11/10, F/M) patients from 21 different families were diagnosed with IEM. The patients were diagnosed with tyrosinemia type 1 (n=4, 19.04%), galactosemia (n=4, 19.04%), Niemann-Pick type A (n=3, 14.28%), glycogen storage disease type 3 (n=2, 9.52%), peroxisomal disorders (n=2,

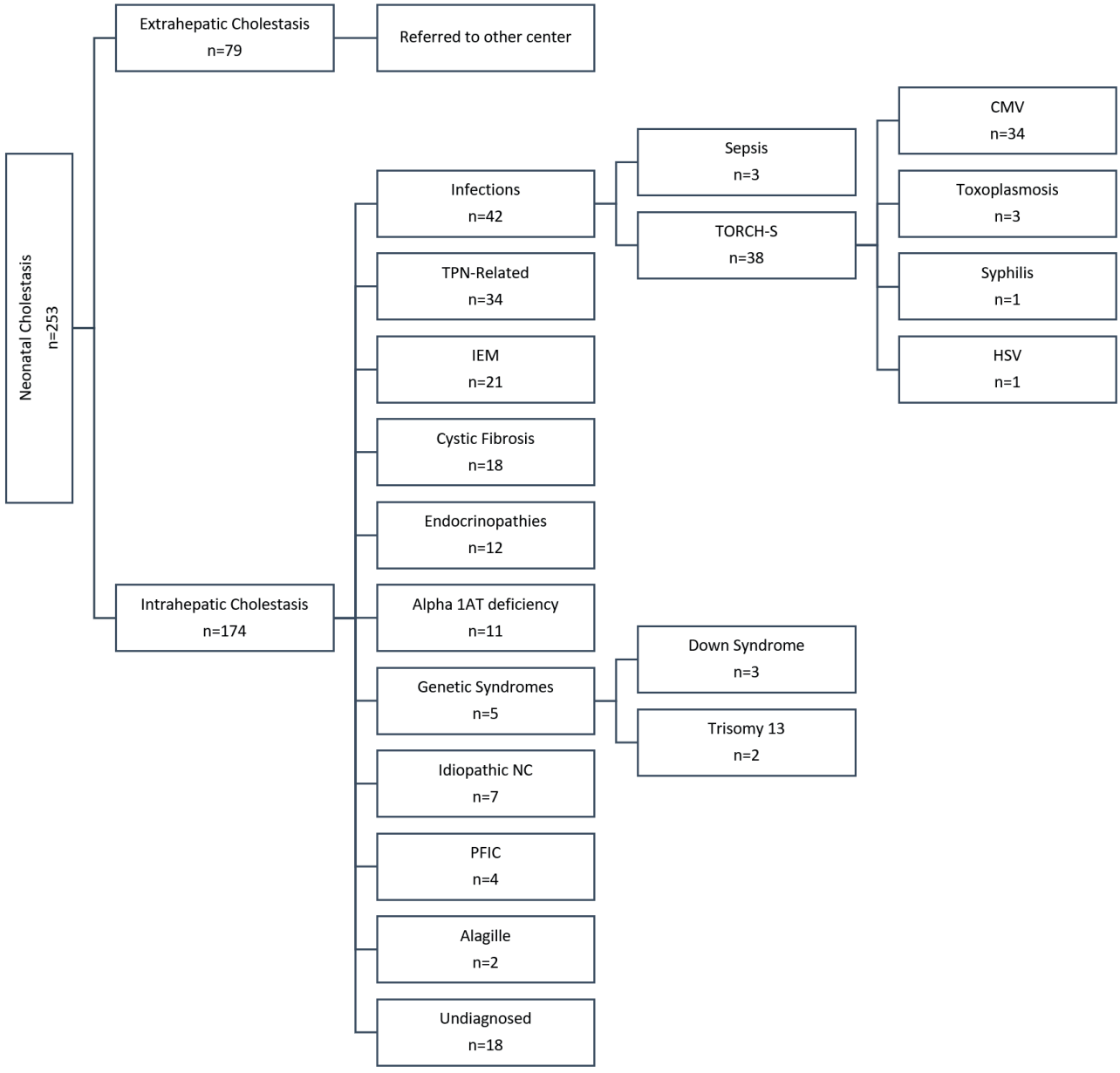

Figure 1. Etiology of the neonatal cholestasis patients in our cohort

9.52%), fatty acid oxidation defects (n=2, 9.52%), mitochondrial DNA depletion syndrome (n=1, 4.76%), citrin deficiency (n=1, 4.76%), Niemann-Pick Type C (NPC, n=1, 4.76%), and bile acid synthesis defect (n=1, 4.76%) in IEM.

We evaluated the patients in two groups, the IEM group and the non-IEM IHNC group. While the consanguinity rate was 90.47% in IEM patients and 25.18% in non-IEM patients. The family history rate was 14.28% in IEM patients, while it was 3.7% in non-IEM IHNC patients. In IEM patients, all patients were born at term with an uneventful delivery, whereas in non-IEM patients, the rate of prematurity was 22.22%, and the rate of hypoxia was 11.85%.

Clinical findings

The mean age of the initial clinical symptom was 38.61±28.38 days (min:7 max:89) and the mean age at the diagnosis was 56.66±34.16 days (min:11- max:112) in the IEM group. The mean age of the initial clinical symptom was 26.42±14.25 days (min:2

max:89) and the mean age at diagnosis was 35.21±22.6 days (min:13- max:108) in the non-IEM group. The most common clinical findings in the IEM group were jaundice (66.66%), hepatomegaly (52.38%), and hypotonia (28.57%), whereas, in the non-IEM group, it was jaundice (92.5%). Hepatomegaly was observed more in the IEM group than in the non-IEM group. Splenomegaly was detected in two patients with Niemann-Pick Type A/B and NPC during the follow-up. Multisystem involvement was observed in 17 (80.95%) IEM patients and 15 (8.62%) non-IEM patients.

Laboratory findings

When ALT values were compared, no statistically significant difference was found between the two groups, however, AST values were significantly higher in the OIHNC group ($p<0.05$). When the bilirubin values of the patients were compared, no significant difference was found between the two groups. A comparison of the findings of IEM and non-IEM intrahepatic neonatal cholestasis patients is shown in Table 1.

Plasma amino acid analysis was performed in 74 patients. There were 21 patients with a diagnosis of IEM and 53 patients with a diagnosis of non-IEM. In the IEM group, 11 patients (52.38%) had elevated plasma tyrosine (Tyr), four patients (19.04%) had low plasma tyrosine, and six patients (28.57%) had normal plasma tyrosine levels. The mean plasma tyrosine levels were 780.18±346.67 µmol/L in patients with elevated Tyr levels and 24.5±4.6 µmol/L in patients with low Tyr levels. Plasma methionine levels were also elevated in eight patients (38.09%), indicating impaired hepatocellular function. Three patients had low methionine levels. Plasma tyrosine and methionine levels were high in patients with not only tyrosinemia type 1 but also galactosemia. When the plasma amino acid results of 53 patients in the non-IEM group were evaluated, high Tyr was found in nine (16.98%) patients, low Tyr in 11 (20.75%) patients, and normal Tyr in 33 (62.26%) patients. The mean plasma tyrosine levels in the OIHNC group were 180.2±42.2 µmol/L in patients with elevated Tyr levels and 11.6±3.4 µmol/L in patients with low Tyr levels. Plasma methionine levels were elevated in two patients (3.77%) in the OIHNC group. Low branched-chain amino acids (one or more from valine, leucine, isoleucine) were detected in nine (42.85%) patients in the IEM group and 13 (24.52%) patients in the non-IEM group. In galactosemia patients, elevations were observed in many amino acids, including Tyr, methionine, and branched-chain amino acids. Hyperaminoacidaemia was transient in galactosemia patients. When liver functions returned to normal, amino acid concentrations returned to normal.

Treatment

Two patients underwent orthotopic liver transplantation. The first patient had tyrosinemia type 1, the other had a bile acid synthesis defect, and both suffered from liver failure. Regarding the outcome, seven patients died in the IEM group. The mean age at death was 6.41±5.74 months (min:1.4- max:18). Therapeutic plasma exchange was performed in two patients with FAO disorders, but had no effect on mortality. The demographic and clinical characteristics of the IEM patients are presented in Table 2.

Table 1. Comparison of the findings of IEM and Non-IEM patients with intrahepatic neonatal cholestasis (OIHNC)

	IEM n=21 (%)	Non-IEM n=135 (%)	p Value
Gender F/M	11/10	72/63	
Consanguinity Rates	19 (90.47%)	34 (25.18%)	p<0.05
Family History	3 (14.28%)	5 (3.7%)	p>0.05
Prematurity rate	0	30 (22.22%)	p<0.05
Birth asphyxia	0	16 (11.85%)	p>0.05
The mean age at initial findings	38.61±28.38	26.42±14.25	p>0.05
Jaundice	14 (66.66%)	125 (%92.5)	p>0.05
Hepatomegaly	11 (52.38%)	28 (20.74%)	p<0.05
Hypotonia	6 (28.57%)	11 (8.14%)	p>0.05
ALT (IU/L)	449.95±512.41	672.3±324.44	p>0.05
AST (IU/L)	628.38±896.63	976.18±726.	p>0.05

Table 2. Demographic, clinical, and molecular characteristics of IEM patients

P	Diagnosis	Sex	Consang.	Family history	Age at Diagnosis	Age at initial Symptom	Initial Symptom	HMG	Hypotonia	Acute Liver Failure	ET	Coag.	Initial Plasma Tyrosine Levels (µmol/L, N:31-108)	Outcome at last visit
P1	Tyrosinemia Type 1	M	Y	N	19	12	ET	Y	N	Y	Y	Y	774	Alive at 2 years of age, under diet and medication
P2	Tyrosinemia Type 1	F	Y	N	22	19	ET	N	N	Y	Y	Y	842	OLT at 5 months of age. Alive at 3 years of age.
P3	Tyrosinemia Type 1	F	Y	Y	19	14	Jaundice	N	N	N	Y	Y	695	Alive at 1 year of age, under diet and medication
P4	Tyrosinemia Type 1	F	Y	N	29	17	Jaundice, elevated Phe levels	N	N	N	Y	Y	554	Alive at 8 months of age, under diet and medication
P5	Galactosemia	F	Y	N	11	7	Jaundice, PC	N	N	Y	Y	Y	856	Alive at 1 year of age, under diet
P6	Galactosemia	M	Y	N	17	11	Jaundice	N	N	Y	Y	N	1108	Alive at 9 months of age, under diet
P7	Galactosemia	F	Y	N	13	9	Jaundice, vomiting	N	N	Y	Y	N	1255	Alive at 8 months of age, under diet
P8	Galactosemia	F	N	N	12	8	Jaundice, ET	N	N	Y	Y	Y	672	Alive at 14 months of age, under diet
P9	Niemann Pick Type A/B	F	Y	N	102	82	AD	Y	N	N	N	Y	28	Deceased at 18 months
P10	Niemann Pick Type A/B	F	Y	N	55	45	Jaundice, hypotonia	Y	Y	N	N	Y	30	Deceased at 92 days of age.
P11	Niemann Pick Type A/B	M	Y	N	72	72	AD, Jaundice	Y	Y	Y	Y	Y	61	Alive at 10 months of age with tracheostomy and gastrostomy

P: patient, F: female, M: male, Consang.: Consanguinity HMG: Hepatomegaly, Coag.: Coagulopathy, ET: elevated transaminases, GSD: Glycogen storage disease, FAO: fatty acid oxidation disorders, Phe: Phenylalanine, PC: poor condition AD: Abdominal distension, OLT: Orthotopic liver transplantation.

Table 2. Continued

P	Diagnosis	Sex	Consang.	Family history	Age at Diagnosis	Age at initial Symptom	Initial Symptom	HMG	Hypotonia	Acute Liver Failure	ET	Coag.	Initial Plasma Tyrosine Levels (µmol/L, N:31-108)	Outcome at last visit
P12	GSD III	F	Y	N	106	84	Hypoglycemia, ET	Y	N	N	Y	N	50	Alive at 1 year of age
P13	GSD III	M	Y	N	109	72	AD, ET	Y	N	N	Y	N	61	Alive at 14 months of age
P14	Peroxisomal Disorders	M	Y	N	69	41	Hypotonia, jaun-dice	Y	Y	N	Y	N	46	Deceased at 5 months of age
P15	Peroxisomal Disorders	M	Y	N	76	17	Jaundice, hypotonia	Y	Y	N	Y	N	71	Deceased at 12 months of age
P16	FAO	F	Y	N	79	61	ET, jaundice	N	N	Y	Y	Y	19	Deceased at 3 months of age due to cardiac arrhythmia
P17	FAO	M	Y	Y	76	63	ET, PC, hypotonia	Y	Y	Y	Y	Y	21	Deceased at 78 days of age
P18	Mitochondrial disease	M	Y	N	37	7	Jaundice, PC, Hypotonia, ET	Y	Y	Y	Y	Y	1342	Deceased at 40 days old.
P19	Citrin Deficiency	F	Y	N	40	20	Jaundice	N	N	N	Y	N	327	Alive at 10 months old
P20	Niemann Pick Type C	M	N	Y	112	89	Jaundice	Y	N	N	N	N	63	Alive at 12 months of age and under medication
P21	Bile acid synthesis disorder	M	Y	N	90	45	Jaundice	N	N	Y	Y	Y	157	OLT at 6 months of age. Alive at 17 months of age.

P: patient, F: female, M: male, Consang.: Consanguinity HMG: Hepatomegaly, Coag.: Coagulopathy, ET: elevated transaminases, GSD: Glycogen storage disease, FAO: fatty acid oxidation disorders, Phe: Phenylalanine, PC: poor condition AD: Abdominal distension, OLT: Orthotopic liver transplantation.

Table 3. Molecular analysis of the IEM patients

Patient	Diagnosis	Gene	Zygosity	Molecular Analysis
P1	Tyrosinemia Type 1	FAH	Homozygous	c.441_448delGGTGATGC
P2	Tyrosinemia Type 1	FAH	Homozygous	c.1062+5G>A (IVS12+5G>A)
P3	Tyrosinemia Type 1	FAH	Homozygous	c.315-3C>G (IVS3-3C>G)
P4	Tyrosinemia Type 1	FAH	Homozygous	c.456-1G>T (IVS6-1G>T)
P5	Galactosemia	GALT	Homozygous	c.563A>G (p.Gln188Arg)
P6	Galactosemia	GALT	Homozygous	c.563A>G (p.Gln188Arg)
P7	Galactosemia	GALT	Homozygous	c.563A>G (p.Gln188Arg)
P8	Galactosemia	GALT	Compound heterozygous	c.563A>G (p.Gln188Arg)/c.958G>A (p.Ala320Thr)
P9	Niemann Pick Type A/B	NA		
P10	Niemann Pick Type A/B	SMPD1	Homozygous	c.967A>C (p.Ser323Arg)
P11	Niemann Pick Type A/B	SMPD1	Homozygous	c.416T>C (p.L139P)
P12	GSD III	AGL	Homozygous	c.500dupG (p.Leu168fs*3)
P13	GSD III	AGL	Homozygous	c.1694delA (p.Asn565MetfsTer12)
P14	Peroxisomal Disorders	PEX	Homozygous	PEX2:c.355C>T (p.Arg119*)
			Homozygous	PEX5:c.159del (p.Glu54Argfs*18)
P15	Peroxisomal Disorders	PEX	Homozygous	PEX1 c.274G>C, p.(Val92Leu)
P16	FAO	ACADVL	Homozygous	c.623G>A (p.Gly208Glu)
P17	FAO	NA		
P18	Mitochondrial disease	TRMU	Homozygous	c.835G>A (p.V279M)
P19	Citrin Deficiency	SLC25A13	Homozygous	c.1793T>G (p.L598R)
P20	Niemann Pick Type C	NPC1	Compound heterozygous	c.2842G>A (p.Asp948Asn)/c.2009G>T (p.Cys670Phe)
P21	Bile acid synthesis defect	AKR1D1	Homozygous	c.148C>T (p.Arg50*)

Molecular analysis

The diagnoses were confirmed by molecular analysis in all IEM patients, presented in Table 3. We studied single gene analysis in 12 (63.15%) patients and multigene panels in seven (36.84%) patients. While single gene sequence analysis was performed in biochemically diagnosed conditions, the multigene panel was preferred when more than one disease was present in the pre-diagnosis. The molecular analysis could not be performed in two IEM patients. P9 was diagnosed by sphingomyelinase enzyme analysis, and P17 was diagnosed by clinical and diagnosed sibling history.

DISCUSSION

Evaluation of patients with neonatal cholestasis is difficult due to the variety of cholestatic syndromes and non-specific clinical findings. Inborn errors of metabolism disorders can produce all the major manifestations of liver dysfunction, such as jaundice, hepatosplenomegaly, coagulopathy, ascites, and encephalopathy. Family and personal medical details should be collected in children with prolonged jaundice and neonatal cholestasis. It is extremely important to take a detailed history and perform a complete physical and neurological examination to formulate possible differential diagnoses. Pregnancy and perinatal history are very important in identifying factors associated with some multifactorial, transient forms of cholestasis, such as low birth weight, prematurity, asphyxia, sepsis, and total parenteral nutrition. Patients with neonatal cholestasis have some markers for diagnosis in their medical history, physical examinations, or basic laboratory tests. Red flags suggesting IEM as the cause of the NC include similar family history, deceased sibling, parental consanguinity, atypical facial appearance, failure to thrive, nystagmus, cataracts, acute or recurrent liver failure, severe hepatomegaly, splenomegaly, cardiomyopathy, dysrhythmia, acute encephalopathy, hypoglycemia, hyperammonemia, rhabdomyolysis, and elevated AFP without hepatocellular carcinoma.[3,7]

Considering the etiology of 253 patients with neonatal cholestasis in our study, intrahepatic cholestasis was detected in the vast majority of cases (68.77%), most of which were infection-related cholestasis (24.13%). Consistent with the literature, the etiology of NC was determined as 60-70% intrahepatic cholestasis. Infection and TPN-related cholestasis constitute the majority of intrahepatic cholestasis cases.[7,8] As a result of clinical and molecular developments in recent years, the frequency of diagnosis of idiopathic neonatal hepatitis has decreased.[3] In the literature, the frequency of NC due to infections has been reported as 7.7%, 2.6%, and 11.5%.[7,9] Consistent with the literature, the most common cause was CMV infection. In Gottesman et al.'s study, the frequency of IEM in the etiology of NC was found to be 4.4%.[7] In the study of Sağ et al., conducted in Türkiye, the frequency of IEM etiology was determined as 12.1%.[9] In our cohort, the frequency of the IEM patients with NC was 8.3%. The higher incidence of IEM can be explained by the fact that consanguineous marriages are more common in Türkiye than in the USA and European countries. Consistent with the literature, the most common causes were galactosemia and HT-1.[7,9]

Sag et al. reported that the frequency of parental consanguinity was 32.8%.[9] In our cohort, the frequency of parental consanguinity was 25.18% in the OIHNC group and 90.47% in the IEM group. The frequency of parental consanguinity was significantly higher in NC patients with IEM etiology. The family history rate was 14.28% in IEM patients, while it was 3.7% in OIHNC patients. If there is consanguinity and/or similar family history in a patient with neonatal cholestasis, it should be carefully evaluated for IEM. The frequency of prematurity was significantly higher in OIHNC patients.

According to the study by Moreira-Silva et al. in 2019, the mean age at presentation of NC patients with only IEM etiology was reported to be 3.5 weeks.[8] According to the study by Sağ et al. in 2013, when all extrahepatic and intrahepatic etiologies were examined, the mean age at presentation in NC patients was reported to be 45±41.3 days.[9] Consistent with the literature, the mean age at first presentation of NC patients with IEM etiology in our study group was 38.61±28.38 days. There was no difference in the mean age at presentation between the patients with IEM etiology and the other groups. Even if NC is a manifestation of liver disease, other systemic diseases and IEMs involving the liver may also present with cholestasis. For this reason, it is recommended to investigate other system involvements. Central nervous system findings and renal pathologies may accompany metabolic diseases.

It is recommended in the NC guidelines to perform metabolic workup, including the second-line workup, in patients suspected as a result of a careful clinical evaluation and initial workup.[10] Second-line workup for IEM can include plasma amino acids, acylcarnitine profile by Tandem Mass Spectrometry, urinary organic acids, and very long-chain fatty acids.[11] Morgan et al. reported significant changes in plasma amino acid concentrations in patients with severe or minimal liver dysfunction.[12] Infants with liver dysfunction usually have low plasma branched-chain amino acid concentrations (valine, leucine, isoleucine) but high phenylalanine, tyrosine, and methionine concentrations.[12-14] Approximately 40% of patients with citrine deficiency have elevated plasma galactose, methionine, and/or phenylalanine concentrations in neonatal screening.[15] However, elevated plasma tyrosine is not common. When the plasma amino acid results of both groups were examined, high Tyr and methionine levels were more common in patients with IEM, in our cohort. Elevated plasma Tyr levels were found in 52.38% of the IEM group and 16.98% of the OIHNC group. Mean plasma tyrosine levels were higher in the IEM group than in the OIHNC group. Plasma tyrosine and methionine levels were high in patients with not only tyrosinemia type 1 but also galactosemia. Hyperaminoacidaemia was transient in galactosemia patients. The frequency of the low branched-chain amino acids was higher in the IEM group than in the OIHNC group. However, the primary factor determining the changes in plasma amino acids is the diagnosis of the disease.

The diagnosis of a treatable IEM causing NC reduces mortality and morbidity. It also helps in genetic counseling, prenatal diagnosis for future pregnancies and clinicians should be alert to the potential associated risk of liver failure and acute metabolic events.

In recent years, NGS-based assays have been developed allowing the simultaneous analysis of multiple genes.[16,17] It has been reported in the literature that NGS-based tests are a promising tool for distinguishing different causes of intrahepatic cholestasis, if the parameters of a reasonable turn-around time, sufficient expertise in the interpretation of results and quality are met and can be considered as a second-line evaluation after exclusion of surgical and infectious etiologies.[10] In our study, we applied single gene analysis in 12 of 19 patients. A specific diagnosis was made with specific biochemical markers and clinical evaluation in 12 patients and the diagnosis was confirmed by single gene analysis. Since there is more than one type and/or gene that causes the disease in patients with peroxisomal disease (2), FAO (1), GSD (2), a disease-associated multigene panel was

performed. However, there were no specific markers in patients with mitochondrial DNA depletion syndrome and even bile acid synthesis defect, the diagnosis was made by clinical exome analysis.

Efficacy data are not available for ursodeoxycholic acid in most of these diseases. However, to promote biliary flow, UDCA was usually prescribed to patients with neonatal cholestasis at doses of 15-20 mg/kg/day. UDCA was generally well tolerated without significant adverse effects. From the early stage of neonatal cholestasis, patients were supplemented with fat-soluble vitamins (vitamins A, D, E, K) to prevent and treat deficiencies. Most of the patients with galactosemia, HT-1, and citrine deficiency responded well to specific treatments. Two patients with FAO, two patients with peroxisomal disorders, two patients with NP-A, and one patient with TRMU died despite supportive treatment due to the lack of effective treatment of the diseases. The diagnosis of a treatable IEM causing NC reduces mortality and morbidity.

Limitations

Since the cases with suspected extrahepatic cholestasis were referred to another center, we did not have information about their etiology. Although there are many studies on the better-known causes of neonatal cholestasis, to the best of our knowledge, this is the first clinical study conducted on the metabolic etiologies of neonatal cholestasis in Türkiye.

CONCLUSION

In the presence of cholestatic jaundice, severe liver dysfunction, acute liver failure, hepatomegaly with hypotonia, and hepatosplenomegaly, careful evaluation for IEM is essential. In conclusion, IEMs are observed more frequently in the etiologies of neonatal cholestasis in Türkiye due to high parental consanguinity and inadequate newborn screening programs. Clinicians should be aware that serious but treatable conditions such as HT1 and galactosemia may present with NC. Consider treatable disorders early, as therapeutic interventions can be lifesaving early in the disease course. It also helps in genetic counseling, prenatal diagnosis for future pregnancies and clinicians should be alert to the potential associated risk of liver failure and acute metabolic events.

Ethical approval

This study has been approved by the Diyarbakir Gazi Yaşargil Research and Training Hospital Clinical Research Ethics Committee (approval date 02/07/2021, number 819). Written informed consent was obtained from the participants.

Author contribution

Surgical and Medical Practices: AEB, FDA, ATÜ; Concept: AEB; Design: AEB; Data Collection or Processing: AEB, FDA, ATÜ, İT, HB; Analysis or Interpretation: AEB; Literature Search: AEB; Writing: AEB, ATÜ. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Supplementary 1

Detailed description of IEM patients

Patients 1-4: Patients presented with neonatal cholestasis and liver dysfunction during the first month of life. Elevated plasma tyrosine levels, and high urinary succinyl acetone suggested the diagnosis of tyrosinemia type 1, confirmed by *FAH* gene analysis in patients. The mean diagnosis age was 28.5±4.27 days and the mean age of initial findings was 19.5±6.57 days. P4 was admitted to the hospital because of high Phe detection in newborn screening. When the patient's Tyr level was high and the conjugated bilirubin was also found to be high. Two patients had generalized aminoaciduria and phosphaturia due to tubulopathy. A Nitisinone and Tyr-Phe restricted diet was administered to the patients. However, P2 underwent OLT at the age of five months despite treatment.

Patients 5-8: These patients presented with a similar presentation of neonatal cholestasis at newborn period. At first admission, raised plasma amino acid levels (particularly Phe, Tyr, and methionine) were remarkable. The diagnosis of galactosemia was confirmed by enzymatic assay of blood cells after a positive Benedict test in urine. All patients received diagnostic confirmation by molecular analysis of *GALT* gene. The mean age of diagnosis and initial findings was 13.25±2.27 days and 8.75±1.47 days, respectively. Liver disease resolved under galactose restriction.

Patients 9-11: Three patients were admitted to the outpatient clinic with organomegaly and jaundice. Two patients had a 'cherry-red spot' in the eye and hypotonia. A rapid progressive course was observed in all NP-A patients. P11 had an acute liver failure at the age of three months. He was alive at 10 months old with poor condition with tracheostomy and gastrostomy. P9-P10 died due to respiratory problems at 18 months and three months of age, respectively.

Patients 12-13: P12-13 were investigated for elevated transaminases. During the examinations, cholestasis, hypoglycemia, and hypertriglyceridemia were detected. Hepatomegaly, found in both cases, ranged from mild to moderate. Based on the present findings, glycogen storage disease was considered in the patients. NGS panel of glycogen storage diseases was studied. P12 was found to have c.500dupG (p. Leu168fs*3) homozygous variant and p13 was found to have c.1694delA (p. Asn565MetfsTer12) homozygous variant in *AGL* gene. The fasting tolerance of the patients with hypoglycemia responded to frequent feeding and modified cornstarch therapy.

Patients 14-15: In the examination of the patients, who presented with cholestasis and severe hypotonicity, dysmorphological findings (high forehead, large fontanelles, flattened face, broad nasal bridge, dolichocephaly) were found. C26:0 (P14: 4.28 µmol/L, P15: 3.75µmol/L N:0,6-1,3) and C26/C22 (P14: 0.28, P15:0.31 N: 0,011-0,026) values were significantly increased in very long-chain fatty acids in plasma. Phytanic acid and pristanic acid were normal. In both cases, the diagnosis of Zellweger syndrome was confirmed. P14 was found to have two different homozygous pathogenic variants in PEX genes (*PEX2*:c.355C>T (p.Arg119*) and *PEX5*:c.159del (p.Glu54Argfs*18)). The parents, who were first-degree cousins, were found to be carriers for both variants. P14 died at the age of five months with acute liver failure and respiratory problems due to severe hypotonia. P15 had c.274G>C, p.(Val92Leu) homozygous variant in the PEX1 gene. Both parents were found to be carriers for the same variant in segregation analysis. P15 died at the age of 12 months due to aspiration pneumonia. Cholic acid therapy was not used in patients with advanced liver disease as it may be harmful.

Patients 16-17: P16 presented with tachycardia, acute liver failure, cholestasis, and rhabdomyolysis. C14-carnitine, C14:1-carnitine, C16-carnitine, C18-carnitine, C18:1-carnitine values were increased, and C0-carnitine (3.77 µmol/L, N:8-20) was decreased in Tandem-MS analysis. Echocardiography revealed hypertrophic cardiomyopathy. The patient had dysrhythmia with various conduction abnormalities and arrhythmias. Sudden cardiac arrest occurred at three months of age. NGS panel of genes associated with fatty acid oxidation revealed homozygous c.623G>A (p.Gly208Glu) variant in *ACADVL* gene. At 2 months of age, P17 presented with hepatic failure, cholestasis, metabolic acidosis, and hyperammonemia that developed during acute bronchiolitis. Fatty acid oxidation defect was considered because the patient had a history of sibling death with the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency (MCAD). A marked increase was found in the concentration of C8-carnitine with the help of tandem mass spectrometry (MS/MS) profile. However, the molecular analysis could not be performed due to healthcare insurance problems. Carnitine supplementation (50 mg/kg/day), liver protective drugs, high dextrose infusion, and ammonia scavengers were initiated. Therapeutic plasma exchange was applied to both patients.

Patients 18: 12 days of age female neonate, was admitted to the hospital in poor condition. Blood tests showed acidosis (pH 7.21), hyperlactacidemia (lactate 5.3 mmol/L), hypoglycemia, and coagulopathy and raised tyrosine and methionine. By the age of 40 days, she developed cholestatic liver disease, hypotonia, and rotational nystagmus. The genetic study confirmed the diagnosis

of mtDNA depletion syndrome with homozygous mutation c.835G>A (p.V279M) in the *TRMU* gene. She died at 3 months old due to acute-on-chronic liver failure.

Patients 19: 2-month-old female patient was admitted to the hospital to investigate the prolonged jaundice and failure to thrive. Elevated transaminases, cholestasis, high levels of alpha-fetoprotein (AFP), and elevated plasma galactose were observed. Enzymatic analysis of blood cells for galactosemia was normal. The diagnosis was confirmed by the detection of a homozygous c.1793T>G (p.L598R) mutation in the *SLC25A13* gene. The patient, who is 10 months old, is under control with lactose-free and MCT-enriched therapeutic formulas.

Patients 20: 89 days old male patient admitted to the hospital because of prolonged jaundice. Liver disfunction, cholestasis and hepatosplenomegaly were detected. Plasma amino acid, carnitine-acylcarnitine, and urinary organic acid analysis were unremarkable. The patients' chitotriosidase activity was high and Sphingomyelinase and beta-glucocerebrosidase enzyme analyses were normal. P20 was found to have compound heterozygous c.2842G>A (p. Asp948Asn) and c.2009G>T (p. Cys670Phe) variants in the *NPC1* gene. The patient was alive at 12 months old and under medication with miglustat.

Patients 21: 45 days old male patient admitted to the hospital with cholestasis. Elevated transaminases, increased AFP, conjugated hyperbilirubinemia, elevated plasma tyrosine, and methionine were detected. Abdominal ultrasonography revealed multiple millimetric calcifications in the liver parenchyma. c.148C>T (p. Arg50*) homozygous variant was detected in the *AKR1D1* gene in multigene panel. The patient had orthotopic liver transplantation at 6 months of age. The 17-month-old patient is being followed up with a mild developmental delay.