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Conjugated hyperbilirubinemia in the neonatal intensive care unit

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Background/aims: *To determine the underlying causes and short-term prognosis of patients with conjugated hyperbilirubinemia in a tertiary neonatal intensive care units.* ***Materials and Methods:*** *We retrospectively analyzed the etiology, course, and short-term prognosis of conjugated hyperbilirubinemia observed in newborn infants in a tertiary neonatal intensive care units.* ***Results:*** *Of a total of 104 infants with conjugated hyperbilirubinemia (2.1%, 104/4915), 92 infants (56 full-term, 36 preterm) were enrolled in the study. Cholestatic jaundice as a sole finding on physical examination during admission was present in 15.2% infants, and conjugated hyperbilirubinemia developed within the first week of life in nearly half of the infants (51.1%). The most frequent causes of conjugated hyperbilirubinemia within the first days of life were vascular/ischemic events, inspissated bile, and inherited metabolic disorders. The majority of the infants (80%) had also concomitant clinical disorders that might possibly contributed to the development of conjugated hyperbilirubinemia. The majority of the deaths (87%) were primarily related to serious perinatal events and genetic/inherited disorders. Bilirubin levels in the most of the survivors (87.1%) returned to normal within six months.* ***Conclusions:*** *Conjugated hyperbilirubinemia is not uncommon in neonatal intensive care unit. Etiology is often multifactorial and more commonly arise from non-hepatic causes. Outcome depends on the underlying causes. Early diagnosis and treatment may be critical for favorable outcome.*

Key words: Cholestasis, etiology, intensive care, newborn

Yenidoğan yoğun bakım ünitesinde direkt hiperbilirubinemi

Giriş ve Amaç: *Yenidoğan yoğun bakım ünitesinde konjüge hiperbilirubinemi saptanan bebeklerde altta yatan nedenlerin ve kısa dönem prognozun araştırılması.* ***Gereç ve Yöntem:*** *Yenidoğan yoğun bakım ünitesinde konjüge hiperbilirubinemi gözlenen bebeklerin dosyaları retrospektif olarak incelendi. Etyolojik nedenler, klinik gidiş ve kısa dönem prognoz açısından tanımlayıcı istatistiksel analiz yapıldı.* ***Bulgular:*** *Konjüge hiperbilirubinemi saptanan 104 (2.1%, 104/4915) bebeğin 92'si (56 term, 36 preterm) çalışmaya alındı. Bebeklerin %15.2'sinde sarılık tek fizik muayene bulgusu idi. Bebeklerin hemen yarısında (%51.1) konjüge hiperbiliruinemi yaşamın ilk haftasında gelişmişti ve bu ilk haftada konjuge hiperbilirubinemi gelişen bebeklerde en sık nedenler vasküler/iskemik olaylar, koyulaşmış safra sendromu ve kalıtsal metabolik hastalıklardı. Bebeklerin çoğunda (%80) konjüge hiperbilirubinemi gelişimine katkısı olabilecek ek klinik durum söz konusu idi. Ölümlerin çoğu (%87) ağır perinatal olay ve genetik/kalıtsal bozukluk ile ilişkiliydi. Yaşayan bebeklerin çoğunda (%87.1) bilirubin seviyesi 6 ay içinde normal sınırlara geriledi.* ***Sonuç:*** *Konjuge hiperbilirubinemi yenidoğan yoğun bakım ünitelerinde ender olmayan bir durumdur. Etyoloji sıklıkla multifaktöriyeldir ve genellikle karaciğer dışı nedenlerden kaynaklanır. Prognoz altta yatan nedene bağlıdır. Erken tanı ve tedavi iyi prognoz açısından önem taşır.*

Anahtar kelimeler: Kolestaz, etyoloji, yoğun bakım, yenidoğan

INTRODUCTION

Hyperbilirubinemia is one of the most common laboratory findings in neonatal intensive care unit (NICU). Clarifying benign and serious causes of hyperbilirubinemia is a critical task faced by clini-

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cians in daily practice (1). Cholestasis is a term described for conditions associated with retention of substances normally excreted into the bile. Conjugated hyperbilirubinemia (CH) that likely reflects serious underlying pathologies is the most common marker of cholestasis (2, 3).

Neonatal cholestasis was reported to occur 1 in 2500-5000 infants, and extrahepatic biliary atresia and neonatal hepatitis were diagnosed in the majority of cases (4-6). The portion of the category known as an idiopathic neonatal cholestasis has decreased with improving ability to diagnose specific conditions over the past two decades (1, 2). The main concern regarding neonatal cholestasis has recently been concentrated on understanding the pathogenesis of cholestatic disease, developing diagnostic tests and therapeutic agents for chronic cholestatic liver disease (2). However, the etiology of cholestasis in newborns at intensive care settings has a spectrum different from that reported in epidemiological studies (4, 6), and is usually on multifactorial basis (7-11). In this population, common perinatal events such as perinatal hypoxia, parenteral nutrition, sepsis, poor enteral feeding, and cardiovascular collapse can contribute to the development of cholestasis (9-19), in addition to the structurally and functionally immature biliary tract (1, 8). Thus, the diagnosis of neonatal cholestasis in this population is one of the most challenging issues.

The purpose of this retrospective study was to determine the underlying causes and outcomes of patients with CH in a tertiary NICU.

MATERIAL and METHODS

Patients and Clinical Data

Medical records of 4915 newborn infants (3617 full-term, 1298 preterm) admitted to our NICU between January 2006 and December 2010 were retrospectively reviewed using the hospital's patient registry and tracking system. CH was defined as direct bilirubin value greater than 1.0 mg/dl if total bilirubin is less than 5 mg/dl or direct bilirubin more than 20% of the total bilirubin if the total bilirubin is greater than 5 mg/dl (3). Infants with CH who had at least two bilirubin measurement 5 days apart were selected for the study. Early demise (first days of admission) without any definitive diagnosis or lack in records was the exclusion criteria. After the study has been approved by the local research ethics committee, clinical data, results of laboratory testing and radiological studies, administered medication, and outcome (death, resolving time or specific treatment) of the patients with CH were recorded from the patients' medical files. The study was performed in accordance with Declaration of Helsinki (20).

In our center, an algorithm using a detailed history and physical examination, haematological, biochemical, metabolic and serological investigations, ultrasound, hepatobiliary scintigraphy (after phenobarbital priming) and liver biopsy, when needed, has been used for investigation of neonatal cholestasis. A final diagnosis, as cause of CH, was made after careful consideration of all available data. The conditions likely to predispose to CH were also recorded.

The patients' birth weight, gestational age, gestational history, intrapartum events (birth asphyxia, haemorrhages, etc.), eventful postnatal course (e.g. assisted ventilation, cardiovascular incompetence and support, exchange transfusion), infection status (e.g. sepsis, urinary tract infection, necrotizing enterocolitis, pneumonia), and parenteral nutrition administration were recorded. The laboratory studies such as total and direct bilirubin levels, liver transaminases, prothrombin and partial thromboplastin time, gamma-glutamyl transpeptidase levels, complete blood count, thyroid function tests, and urine analyses were evaluated in all patients. If available, results of the metabolic screening, TORCH battery of tests (toxoplasmosis, rubella, cytomegalovirus (CMV), herpes virus, syphilis) , serum alpha-1 antitrypsin (AAT) levels, sweat chloride test, radiological studies (ultrasonography, hepatobiliary scintigraphy, echocadiography), and liver biopsies were also evaluated.

Definitions

The presumptive diagnosis of biliary atresia was made by liver biopsy (7), and confirmed by intraopreative cholangiography. The diagnosis of neonatal hepatitis syndrome including intrauterine infection, genetic syndromes, endocrine disorders, and inborn errors of metabolism was based on clinical and laboratory data and nonspecific hepatic inflammation in liver biopsy (1,7). Idiopathic neonatal cholestasis was described when CH persisted beyond three months of life, and any other identifiable causes could not be determined (1,7). Bile duct paucity is defined histologically in a full-term or older infant as a ratio of bile duct to por-

tal tract that is less than 0.9 in a minimum of ten portal tracts (1). Inspissated bile syndrome was considered in neonates with relevant history as presence of severe unconjugated hyperbilirubinemia requiring exchange transfusion and ultrasound findings including bile sludge and/or bile duct dilatation, and/or improvement with the ursodeoxycholic acid (UDCA) therapy (7). Parenteral nutrition-associated cholestasis was a clinical diagnosis based on the use of total parenteral nutrition (TPN) for two weeks or longer before onset of cholestasis in the absence of any other identifiable cause, and gradually improvement after discontinuation of TPN (19).

While the causes of CH were classified, the vascular group (or systemic disorder) was defined as conditions such as perinatal asphyxia, septic shock, congenital heart defects (CHD) leading to severe hypoxia and hypotension. As a final diagnosis, category named "unknown" included patients whose CH resolved during follow-up and required no more investigation or who died without any specific diagnosis.

Statistical Analysis

Analyses were performed using the SPSS for Windows (version 16.0; SPSS Inc., Chicago, IL). The numeric data were expressed as mean (SD) or median (min-max) in respect to variables which were normally distributed or not, respectively. The categoric variables were shown as number and percentage.

RESULTS

Demographic Features and Clinical Findings

A total of 104 infants with CH (2.1%, 104/4915) were identified. Of them, those who died on first days of admission (n: 7) and who had irregular visits (n:5) were excluded. Remaining 92 infants (56 full-term, 36 preterm) showed slightly male (56.5%) predominance. Seventeen preterm infants (47.2%) had very low birth weight (VLBW), and 14 infants (15.2%) were small for gestational age (SGA). The mean gestational age of the premature infants was 31.3 (3.2) weeks (range: 26-36 weeks), and the mean birth weight was 1630 (631) g (range: 800-2900 g).

Cholestatic jaundice on physical examination during admission was present in 14 (15.2%) infants, and in the remaining, CH was detected during hospital course. The mean postnatal age at diagnosis of CH was 14.3 (16.9) days (range: 1-65 days). CH developed within the first week of life in nearly half of the infants (51.1%, 47/92). The most common causes of CH in this group of infants were inspissated bile (n:16), perinatal asphyxia (n:9), inherited metabolic disorder (n:5) [familial hemophagocytic lymphohistiocytosis (FHL), hydroxymethylglutaryl (HMG) CoA lyase deficiency, citrullinemia, methylmalonic acidemia (MMA), galactosemia], and CHD (n: 5). Two infants diagnosed with bile duct paucity also presented with CH within first days of life.

CH was associated with hepatomegaly in 14 infants which is defined as liver edge > 4 cm palpable below the costal margin. Of them, 6 had metabolic disorder (2 with galactosemia, and each one with HMG CoA lyase deficiency, citrullinemia, MMA, and FHL). Three had inspissated bile (one with rhesus disease, one with spherocytosis, one with pyruvate kinase deficiency), two had intrauterine infection (syphilis and CMV hepatitis), and each one of the remaining had biliary atresia, idiopathic neonatal hepatitis, and bile duct paucity.

Persistent acholic stools were detected in 4 infants with biliary atresia, while infants with bile duct paucity (n:3) showed intermittent feature. Four infants (4.4%) presented with bleeding (three with hematemesis, one with intracranial bleeding). Two of them had biliary atresia, one had idiopathic neonatal hepatitis, and one had galactosemia.

Hepatobiliary scintigraphy was performed in 26 infants with acholic stools (either intermittent or persistent), whose diagnosis was inconclusive after the baseline investigations or liver histology was unequivocal. Hepatobiliary scintigraphy failed to show biliary excretion into the gastrointestinal tract in 7 of 26 infants (Figure 1).

Liver biopsies (n: 12) showed extrahepatic biliary atresia (n:4), idiopathic neonatal cholestasis (n: 4), bile duct paucity (n: 3), and TPN-associated cholestasis (n: 1).

Underlying Causes of CH and Outcome

The final diagnosis of underlying causes of CH is given in Table 1. The majority of infants (80.4%, 74/92) had also concomitant clinical disorders that might have contributed to the development of CH (Table 2).

i. Vascular/ischemic disorder

The vascular/ischemic pathologies were the leading cause (n: 28, 30.4%) of CH. The frequencies of

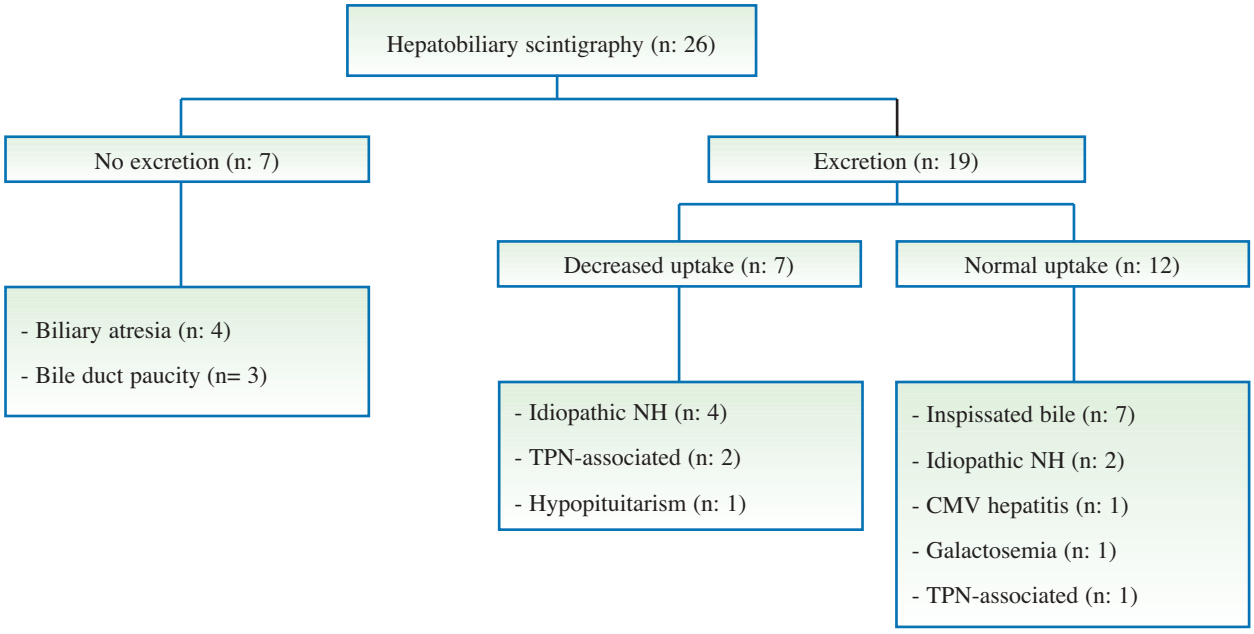

Figure 1. Hepatobiliary scintigraphy results and final diagnosis in the patients with conjugated hyperbilirubinemia.
TPN, topal paranteral nutrition; NH, neonatal hepatitis; CMV, cytomegalovirus

Table 1. Etiology of conjugated hyperbilirubinemia in 92 infants

Underlying cause	No. (%)	Final diagnosis (n) (n*)
Ischemia†	28 (30.4)	Perinatal asphyxia (11) (8*), Congenital heart disease (9) (6*), Septic shock (8)(5*)
Inspissated bile	21 (22.8)	Blood group incompatibility (Rh, ABO) (13), non-immune hemolytic anemia (2), Down syndrome (1), dehydration (1), bilateral adrenal hemorrhage (1), unknown (3)
Metabolic/genetic	11 (12.0)	Galactosemia (3), hypothyroidism (3), hypopituitarism (1), HMG CoA lyase deficiency (1), citrullinemia (1) (1*), methyl-malonic acidemia (1) (1*), FHL (1) (1*)
Infectious	9 (9.8)	Candidemia (3), CMV hepatitis (2), pneumonia (2), congenital syphilis (1), nosocomial sepsis (1)
Toxic	8 (8.7)	Total parenteral nutrition (8) (4*)
Idiopathic	7 (7.6)	Idiopathic neonatal cholestasis (7) (1*)
Biliary obstruction	5 (5.4)	Biliary atresia (4) (1*), annular pancreas (1)
Bile duct paucity syndromes	3 (3.3)	Non-syndromic (2) (1*), ARC syndrome (1) (1*)

* Number of cases died; † Conditions leading to severe hypoxia and hypotension
CMV: Cytomegalovirus; FHL: Familial hemophagocytic lymphohistiocytosis; HMG: Hydroxy methylglutaryl; ARC: Arthrogryposis, renal tubular dysfunction and cholestasis

underlying causes of vascular/ischemic pathologies and mortality rate are shown in Table 1. It is noteworthy that all infants in this group were referred to our unit from another center. Clinical and laboratory findings of all these infants indicated multi-organ failure (MOF). All of them required invasive assisted ventilation and cardiovascular support including volume expanders and inotropic agents. Liver enzymes elevated more than 10 times of upper limit and coagulopathy over the reference range for age were found in the majority of them (24/28 and 21/28, respectively). *Escherichia coli* was the most frequently isolated microorganism in septic infants (55.6%, 5/9). Nineteen of

Table 2. Contributory factors associated with the development of conjugated hyperbilirubinemia other than primary causes in 74 infants†

	Number	Frequency
Infection‡	18	24.3
Low birth weight (< 1500 g)	17	23.0
Respiratory distress/Hypoxia§	16	21.6
Cardiac pathology (except ASD)	15	20.3
Small for gestational age	14	18.9
Portal vein thrombosis	10	13.5
Exchange transfusion	9	12.2
Parenteral nutrition¶	9	12.2
Hypothyroidism	5	6.8
Trisomy 21	4	5.4

†: The conditions occurred within 7 days before onset of or during the course of conjugated hyperbilirubinemia; ‡: Sepsis, urinary tract infection and necrotizing enterocolitis; §: Disorders requiring assisted ventilation, ¶: The infants who received total parenteral nutrition for at least 7 days; (*5, 4, 3 and 2 factors were coexistent in 4, 10, 14 and 18 infants, respectively).

28 infants (67.9%) died due to MOF within one month, and the remaining 9 infants (32.1%) recovered completely with appropriate treatment, and direct bilirubin levels returned to normal within two weeks.

ii. Inspissated bile

Inspissated bile was the second most prevalent (n: 21, 22.8%) cause of CH. Frequencies of underlying causes of inspissated bile are shown in Table 1. All of these infants undergone one or more exchange transfusion which was followed by CH. Five infants had also complicated with portal vein thrombosis (probably due to umbilical venous catheterization for exchange transfusion) that was resolved by appropriate treatment in all. Ultrasonographic examination revealed biliary sludge (seen in gall bladder) in 15 infants (71.4%) and bile duct dilatation (also biliary sludge in common bile duct) in 3 infants (14.3%). The remaining infants had normal ultrasonographic findings (the diagnosis of inspissated bile in these infants was based on clinical course). Hepatobiliary scintigraphy performed in 7 infants was found as normal. Of them, 3 (14.3%) had also elevated GGT levels. Except these 3 infants, CH resolved within one month in the remaining patients. In these 3 infants who had high GGT levels, CH persisted for 3 months; and they were diagnosed with rhesus disease, hereditary spherocytosis, and pyruvate kinase deficiency. The patients with persistent CH more than two weeks (23.8%, 5/21) received UDCA. None of the infants required invasive intervention.

iii. Metabolic and genetic

A metabolic or genetic cause was identified in 11 infants. Three patients with galactosemia had a favorable outcome on follow-up, while each one of the infants with MMA and urea cycle defects (citrullinemia) died due to catastrophic metabolic decompensation and hepatic failure, respectively. One infant with FHL died in the second week of life. Another infant who presented with clinical sepsis during neonatal period and was later diagnosed with HMG CoA lyase deficiency at 5 months of life revealed a favorable outcome with dietary treatment. CH was resolved by hormone replacement therapy in 3 infants with hypothyroidism and in one with panhypopituitarism.

iv. Infectious

The infectious cause was identified in 9 infants and differed from the vascular group by the absence of severe hypotension and hypoxia. Three preterm infants with candidemia were under parenteral nutrition as well. Three infants had intrauterine infection with CMV (n:2) and syphilis (n:1), respectively; the latter one having hydrops fetalis. Two infants had nosocomial infection: *Klebsiella pneumonia* (n:1), and Pseudomonas aeruginosa (n:1). One infant had community-acquired pneumonia of which the causative agent was not identified. All infants survived with appropriate treatment. CH resolved within one month in 7 infants, while in those with CMV hepatitis and congenital syphilis, it persisted until 4 and 7 months of age, respectively.

v. Idiopathic

The etiology of CH could not be determined in 7 infants. Five of them were preterm, and the remaining 2 were SGA. Of 2 with SGA, one presented with hydrops and died by 54 days of age, and the other one was well by 35 days of age. Cholestasis resolved within six months in 4 infants. CH persisted over one year without any other clinical and biochemical abnormalities of liver disease in one term infant who had undergone an exchange transfusion during the neonatal period. She is the first child of a consanguineous parents, and 2 years old now. The cause of CH in this infant has not been clarified yet. All these infants received supportive nutritional management (including fat-soluble vitamins) and UDCA until resolution of cholestasis.

vi. Bile duct paucity

Nonsyndromic bile duct paucity was diagnosed in 3 infants. All of them were full-term, and CH was detected within the first week of life. Jaundice, hepatomegaly, and recurrent acholic stools were the most prominent findings in these infants. One infant was diagnosed with arthrogryposis, renal tubular dysfunction and cholestasis (ARC) syndrome. His sibling had also ARC syndrome. Both died within the first six months of their life. In the second infant, hypothyroidism and urinary tract infection were present concomitantly, but CH did not resolve by appropriate treatment of these disorders. She died of hepatic failure at one year of age. In the third infant, clinical and biochemical abnormalities of liver disease persisted over one year. She is now two years old and still on follow-up. All infants received dietary supplements and UDCA therapy.

vii. TPN-associated cholestasis

Of the 92 study patients, 32 received TPN, and 13 of them required TPN for longer than 14 days. TPN-associated cholestasis was defined in 8 infants by excluding the other causes. All of them were preterm and VLBW, except one. The median duration of TPN administration was 36 days (range:15-60 days). Of them, 4 patients died due to overwhelming infection without resolution of the cholestasis. In the remaining 4 survivors, cholestasis resolved within six months after cessation of parenteral nutrition.

viii. Extrahepatic biliary obstruction

Biliary atresia was diagnosed in 4 out of 5 patients. Two of them were preterm born at 34th week of gestation. In these 2 infants, CH was detected within the first two weeks of life. Of them, one presented with apnea secondary to supraventricular tachycardia, and CH was detected at age of 8 days. The other 2 infants presented with bleeding (intracranial and gastrointestinal) without acholic stools. Sera from these 4 infants were unremarkable for CMV infection. The fifth infant who presented with duodenal obstruction was diagnosed with annular pancreas, which complicated with obstructive jaundice in due course. Of the 5 infants, 4 revealed a favorable outcome after surgery. One patient with biliary atresia died 5 days after surgery performed at 50 days of age. CH with slightly elevated transaminases, without clinically jaundice and acholic stool, persisted over one year in two of the survivors. They are two and three years old now and are on follow-up in gastroenterology unit. None of the infants required liver transplantation yet.

DISCUSSION

There are many causes of CH in newborn infants admitted to NICU (8-11). The immaturity of hepatobiliary system makes newborn infants relatively more susceptible to a wide variety of disorders (1,21). As seen in the present study, a substantial number of cases are associated with perinatal events which are possibly responsible for chronic or acute perinatal distress (10,15), while others probably have a primary disease of the liver (15,22). The present study was intended to evaluate the underlying causes and outcome of patients with CH in a NICU and to try to understand "What's happening to these cases?". Unlike to some previous reports (3,21,23,24), this study included only newborn infants with CH, and observation period was limited to one year period. Hence, the results may be different from those of outpatient settings (3,21,23,24), and might be insufficient regarding long-term prognosis. In contrast to previous studies (9,11,14) which report preterm predominance, only 35% of patients were premature in this series. This might also have impact on the distribution of the etiology of the CH.

The most common causes of CH in this series were ischemic etiologies and inspissated bile in order, and they were distinct in patterns. The first was characterized with cardiovascular failure, which was resulted from variety of disorders including asphyxia, shock and heart defects. Several previous studies have also identified these entities as an important causal factor in hepatic dysfunction (10,12-18,25,26). Such dysfunction may be explained by the theory of hepatic cell integrity and bile secretion processes which are impaired by hepatic hypoxia-ischemia (10). However, it is thought to be of multifactorial origin (13). Liver injury in such cases seems to be a part of multisystem organ involvement. The severity and persistence of liver injury depend on underlying disorders, and the liver damage is usually reversible by stabilization of circulatory problem (25,26). However, normalization of liver dysfunction may take several months (12,14). Because such early-onset CH is due to non-cholestatic liver diseases, it does not usually warrant invasive investigation. The high rate of these disorders in our series may be the result of our center's status as a referral community hospital.

The second cause was inspissated bile which is often secondary to the increased demand on the biliary system from excessive bilirubin load (8), and is usually associated with a benign clinical course (27). It is thought that the excessive bilirubin load presented to the liver causes blockage of the ducts during excretion (28). In fact, this entity is associated with evidence of hepatocellular dysfunction with or without canalicular obstruction (28), but it is classified as "bile duct abnormality" (3,6,8). While the majority of cases have been previously described with a hemolytic disease of the newborn (28), one third of infants in this group did not have any hemolytic disease. The high frequency of inspissated bile in our series may be as a result of the inclusion criteria. The use of umbilical venous catheter for exchange transfusion may also be a contributory factor (9). The presence of hepatomegaly seems to be related to extramedullary hematopoiesis or prolonged CH. In case of severe haemolysis, CH is likely to be early-onset and persistent. However, as shown in the present study, CH often resolves within a month. Thus, invasive investigations in such cases without other clinical and biochemical abnormalities of liver disease could be delayed until a reasonable period.

Sepsis is a well-known cause of neonatal cholestasis (1,15-17,29). It is considered that proinflammatory cytokines play a key role in the pathophysiology (1), and hepatocellular dysfunction has been demonstrated to occur early after the onset of sepsis (29). Liver damage may also occur as a consequence of hypotension or prolonged hypoxia in the course of sepsis if cardio-circulatory failure develops (29). CH is more commonly associated with Gram-negative bacteremia (15-17), however it can occur in case of Gram-positive bacteremia or non-bacterial infection as well (29). Our series also showed that sepsis-induced cholestasis was associated with dominantly Gram-negative agents. Like observed in our cases, cholestasis usually subsides slowly after resolution of the infection (15-17,29).

A considerable proportion of liver disorders with CH were caused by inborn errors of metabolism, and this may be explained by the high incidence of consanguineous marriages in Turkey (30). Since many metabolic disorders have favorable outcome by early treatment, they should be kept in mind for any critically ill newborn infants. An interesting finding in this series was that AAT deficiency was not determined, whereas it is reported to account for approximately 13% of all cholestatic infants (4,22). This may be due to low carrier count in the Turkish population, although there is no available data, and also phenotype determination is not routinely used beyond serum ATT level measurement and histological examination of liver tissue. In a study of 50 infants with neonatal cholestasis from Turkey (31), only one case was found to have serum AAT deficiency and related findings on liver biopsy. The differences in the incidence of genetically associated diseases in our population may affect distribution of the etiology of neonatal cholestasis.

There are different opinions among neonatal units regarding the extensive investigation of CH in NICU settings, particularly in preterm infants. Some authors (9,15) suggest that CH in preterm infants, it is often transient and acquired and does not warrant invasive investigation. On the other hand, the permanent causal factors requiring urgent medical or surgical intervention were documented in selected groups (22). In the present study, although transient causes of CH were determined in most of the preterm infants, extrahepatic biliary atresia was also defined in 2 preterm infants. Therefore, it would be prudent to obey classical algorithm using stool color, ultrasound, hepatobiliary scintigraphy, and liver biopsy for investigation of the CH (7).

Concordant with previous reports (7-11,18), the present study also showed a multifactorial basis for the development of neonatal cholestasis, particularly in preterm infants. In addition to prematurity, these factors were LBW, infection, hypoxemia, hypotension, parenteral nutrition, exchange transfusion, hypothyroidism, cardiac pathologies (mostly ductus arteriosus) and poor enteral feeding. It is often impossible to ascertain whether some factors are causal, contributory, or coincidental. In this study, the most possible etiological factor was defined as final diagnosis. In some situation, such as early death, the definitive final diagnosis could not be made because other potential causative or contributory factors, such as intrauterine infection and inherited metabolic disorders, could not be exactly ruled out. Moreover, biliary duct anomalies can be associated with various types of congenital heart disease (32). In the present series, the underlying etiology might have been masked by the dominant disorder. As a result, the diagnosis of one disease does not preclude the presence of another (3).

Of infants with biliary atresia, two presented with bleeding and one with apnea secondary to cardiac arrhythmia. CH was detected during their hospital course before overt jaundice. We thought that diagnosis of biliary atresia in the latter case was coincidental, whereas arrhythmia-associated cholestasis was also defined previously (33). Therefore, in a newborn infant admitted with unexpected clinical presentation, a careful laboratory and radiological investigations are mandatory. Consistent with our results, considerable proportion of infants with biliary atresia have been reported to have early onset of cholestasis within the first two weeks of life (23,24). Hepatobiliary scintigraphy also makes a remarkable contribution to differential diagnosis especially in differing abnormal excretion and liver uptake patterns (3,7). However, there is a tendency that hepatobiliary scintigraphy adds little to the routine evaluation of the cholestatic infant (6). A study showed that 50% of patients with bile duct paucity but no extrahepatic obstruction failed to show biliary excretion of radionuclide. Twenty-five percent of patients with idiopathic neonatal hepatitis demonstrated no biliary excretion (34). In the present study, 7 out of 26 patients had no excretion of tracer, and scintigraphy was valuable to rule out biliary atresia. However, it is not so specific in predicting biliary atresia, because 3 (42.8%) out of 7 patients with no excretion of tracer were eventually diagnosed as bile duct paucity syndrome.

In this study, only 13% of cases underwent successful liver biopsy. This low rate is a reflection of etiology of CH associated with non-cholestatic liver disease in the majority of cases which does not necessitate histological evaluation. Possibility of inconclusive liver biopsy in small infants might also prompt a conservative approach to invasive investigations, which in case of biliary obstruction is not likely. However, liver biopsy remains the gold standard investigation for differentiating between the several causes of cholestasis (1-3,6,35).

Disorders that require intensive care may be complicated with CH. Unlike the classical cholestasis, etiology is often multifactorial and more commonly arises from the non-hepatic causes. The difficulty of defining the underlying cause of CH because of the superimposing conditions during intensive care requires a careful work-up and follow-up. Since the outcome of the patients depends on the underlying etiology, early detection of primary disorder (especially inherited metabolic disorders affecting the liver, infections, and biliary atresia) and initiation of appropriate treatment are critical for favorable outcome.

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