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Infants with Cholestasis: Diagnosis, Management and Outcome

İnfantlarda Kolestaz: Tanı,Tedavi ve Prognoz

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Abstract

Objective: Infants with cholestatic jaundice were evaluated retrospectively in terms of etiologies, diagnostic methods, laboratory findings, treatment procedures and long- term prognosis.

Patients and Methods: The study consisted of 70 children (52.8% male, 47.1% female) with cholestasis ranging in age from 15 days to 8 months (mean age, 60±26 days). Patients were divided into three groups according to the diagnosis: (i) patients with extrahepatic biliary atresia, (ii) patients with intrahepatic biliary hypoplasia, and (iii) patients with hepatocellular disease. Their clinical parameters were evaluated.

Results: In the group with extrahepatic biliary atresia the onset of jaundice was significantly earlier and the presence of acholic stool and total bilirubin levels were remarkably higher than in the groups with intrahepatic biliary hypoplasia or hepatocellular disease. Serum gamma-glutamyl transpeptidase (GGT) and alkaline phosphotase (ALP) levels were found to be significantly higher in the groups with extrahepatic biliary atresia and intrahepatic biliary hypoplasia than the group with hepatocellular disease ($p<0.001$ and $p<0.01$, respectively). The contribution of technetium-99m (99mTc) scintigraphy to the diagnosis was significantly higher in the group with extrahepatic biliary atresia than the groups with intrahepatic biliary hypoplasia and hepatocellular disease ($p<0.002$).

Conclusion: It was found that cholestasis, acholic stool and elevated GGT are better markers for extrahepatic biliary atresia than for intrahepatic biliary hypoplasia or hepatocellular disease in infants. The contribution of scintigraphy to the diagnosis was found to be higher in the group with extrahepatic biliary atresia than in the other groups. *(Marmara Medical Journal 2012;25:83-6)*

Key Words: Biliary atresia, Hepatocellular disease, Neonatal cholestasis, Biliary hypoplasia, Jaundice

Özet

Amaç: Kolestaz nedeniyle takip edilen çocuk hastalarda etyoloji, tanı yöntemleri,laboratuvar bulguları,tedavi şekilleri ve uzun dönem prognozları açısından geriye dönük olarak incelenmesidir.

Hastalar ve Yöntem: Çalışmaya 15 gün-8 ay (ortalama yaş, 60±26 gün, %52,8 erkek, %47,1 kız) kolestaz tanısı ile izlenmiş olan 70 vaka dahil edildi. Hastalar, (i) ekstrahepatik, (ii) intrahepatik biliyer atrezili hastalar, intrahepatik biliyer hipoplazi (iii) hepatoselüler hastalığı olanlar olmak üzere 3 gruba ayrıldı. Klinik parametreleri değerlendirildi.

Bulgular: Ekstrahepatik biliyer atrezili hasta grubunda diğer gruplara kıyasla sarılığın başlangıç zamanı belirgin olarak daha erken, total bilirubin düzeyi daha yüksek ve akolik dışkı görülme sıklığı daha fazla saptandı. Serum gama-glutamil transpeptidaz (GGT) ve alkalin fosfotaz (ALP) düzeyleri ekstrahepatik biliyer atrezi ve intrahepatik biliyer hipoplazi gruplarında hepatoselüler hastalığı olan gruba göre daha yüksek saptandı ($p<0.001$ ve $p<0.01$). Sintigrafinin tanıya katkısı ekstrahepatik biliyer atrezi grubunda intrahepatik biliyer hipoplazi ve hepatoselüler hastalığı olan gruplara göre daha anlamlı saptandı ($p<0.002$).

Sonuç: Kolestaz, akolik dışkı ve yüksek GGT düzeyleri ekstrahepatik biliyer atrezili vakalarda, intrahepatik biliyer hipoplazi ve hepatoselüler hastalığı olan vakalara göre daha iyi belirteçlerdir. Technetium-99m (99mTc) ile yapılan sintigrafinin tanıya katkısının ekstrahepatik biliyer atrezi grubunda diğer gruplara göre daha belirgin olduğu görüldü. *(Marmara Üniversitesi Tıp Fakültesi Dergisi 2012;25:83-6)*

Anahtar Kelimeler: Biliyer atrezi , Hepatoselüler hastalık, Neonatal kolestaz, Biliyer hipoplazi, Sarılık

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Introduction

Cholestasis is defined as reduced bile flow and abnormal accumulation of conjugated bilirubin, indicating impaired hepatobiliary function. Obstructive (extrahepatic, intrahepatic) or hepatocellular (infectious, metabolic, toxic, genetic or idiopathic) diseases may cause cholestasis in newborns and infants[1-5]. Although extrahepatic biliary atresia (EHBA) and neonatal hepatitis constitute a major part of the cholestatic diseases, it is difficult to make a differential diagnosis in cases with severe neonatal hepatitis mimicking EHBA[3,6,7].

Therefore, further studies are being conducted to use more effective methods in the differential diagnosis of neonatal cholestasis[6-9].

Early detection is essential to facilitate timely intervention and minimize adverse outcomes in several conditions, including biliary atresia, hypothyroidism, and galactosemia. Thus, a systematic approach is helpful to establish the diagnosis quickly. Even when treatment is not available and effective, infants who have progressive liver disease benefit from optimal nutritional support and medical management of the complications of cholestasis and possibly cirrhosis.

The aim of this retrospective study is to evaluate 70 cases with cholestasis in the neonatal period and in infancy in terms of etiologies, diagnostic methods, treatment modalities and the relationship of these with prognosis.

Patients and Methods

Seventy infants with neonatal cholestasis diagnosed between 1998 and 2004 at the Department of Pediatric Gastroenterology of Sisli Etfal Training and Research Hospital Istanbul, Turkey were evaluated retrospectively. Neonatal cholestasis was defined as prolonged conjugated hyperbilirubinemia concentration in a neonate at a level above 1.0 mg/dl where the total serum bilirubin is <5.0 mg/dl, or greater than 20 percent of the total serum bilirubin where the total serum bilirubin is >5.0 mg/dl.

Patients were divided into three groups according to the diagnosis: (i) patients with EHBA, (ii) patients with intrahepatic biliary hypoplasia (IHBH) and (iii) patients with hepatocellular disease (HCD). Significantly low birth weight for gestational age, the onset time of jaundice, and the presence of acholic or hypocholic stools were recorded. Hemoglobin levels, white blood cell counts, levels of alanine transaminase, gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), acid phosphatase, total bilirubin and direct bilirubin, total protein, albumin, globulin, alpha 1-antitrypsin were examined in all cases. Also serological tests for hepatitis B, hepatitis A, toxoplasma, rubella, cytomegalovirus (CMV), herpes simplex (TORCH) and Epstein Barr virus infections, Venereal Disease Research Laboratory (VDRL), blood and urine tests for amino acids, sweat chloride test, thyroid function tests and abdominal ultrasonography were done.

Bone marrow aspirates were examined to rule out storage diseases. A closed needle liver biopsy using a Menghini needle was performed in sixty-six cases under intravenous sedation with midazolam (0.1 mg/kg). Informed consents were taken from all of the parents before the biopsy. If histopathological examination of the liver revealed the presence of bile duct proliferation, periportal fibrosis and bile duct obstruction, the condition was described as EHBA. If the ratio of the number of bile ducts to the number of portal fields was < 0.5, then it was described as IHBH, and HCD was diagnosed by the presence of a lobular disorder, giant cell formation, portal inflammation and minimal fibrosis. Thirty-one cases underwent biliary tract scintigraphy with 99mTc, and excretion into the intestine observed during hepatobiliary scintigraphy was used to rule out EHBA.

All the cases received a formula rich in medium-chain fatty acids in addition to the maternal milk, but those diagnosed as metabolic diseases received a specific formula and vitamins A, D,E and K. Patients with cholestasis were started on ursodeoxycholic acid (10 mg/kg/day). Perioperative cholangiography was done to confirm the diagnosis of EHBA and a Kasai operation was performed to establish bile drainage in these patients. If bile flow is not restored by the Kasai procedure or if life- threatening complications of cirrhosis ensued then a liver transplantation was considered.

Statistical Analysis

The statistical analysis was performed by the program SPSS 11.0 (Chicago, IL, USA). The chi-square test, Fisher's exact test, ANOVA (Tukey-Kramer Test) were used. A value of $p<0.05$ was considered statistically significant.

Results

The age of patients ranged from 15 days to 8 months (mean, 60±26 days), and the male: female ratio (37 male, 33 female) was 1.12. The history of 30 patients (42.8%) revealed parental consanguinity and four (5.7%) were sibling deaths. All of the patients were born at term, and 12 had low birth weights.

Fourteen of the patients (20%) had EHBA, six (9.2%) had IHBH and 50 (71.42%) had HCD. In the EHBA group, two had a CMV infection and one had a hepatitis B infection. In the HCD group, 11 (22%) patients had idiopathic neonatal hepatitis (INH), 22 (44%) had CMV hepatitis, four had tyrosinemia, four had septicemia, two had Niemann-Pick disease, one had hepatitis A, one had hepatitis B, one had a congenital rubella infection, one had alpha 1-antitrypsin deficiency, two had galactosemia and one had hemochromatosis. No case of syndromic variety was diagnosed. No relationship was established between the age of patients at admission to hospital and the rate of splenomegaly. Portal hypertension was not detected in any patient. The clinical and laboratory findings are shown in Tables I and II.

The onset time of jaundice was significantly earlier for EHBA than for HCD ($p<0.05$). The presence of acholic stool was remarkably higher in the EHBA group. Serum GGT and ALP levels were significantly elevated for EHBA and IHBH ($p<0.001$ and $p<0.01$, respectively). Although CMV IgM was positive in all patients diagnosed as CMV hepatitis, CMV DNA was detected in only 12 patients. Increased serum phenylalanine and tyrosine levels were documented in four patients, and succinylacetone was

Table I. Clinical Findings in Patients with Neonatal Cholestasis

	HCD (n=50)	EHBA (n=14)	IHBH (n=6)	Total (n=70)
· Jaundice	44 (88%)	14 (100%)	4 (66.6%)	62 (88.5%)
·Onset time	15.9*	4.5*	11*	17.6 (1-99) jaundice(days)
·Acholic stools	16 (32%)**	13 (92.8%)**	3 (50%)**	32 (45.7%)
·Hepatomegaly	38 (76%)	14 (100%)	6 (100%)	58 (82.8%)
·Splenomegaly	33 (66%)	11 (78.5%)	2 (33.3%)	46 (65.7%)

HCD: hepatocellular disease, EHBA: extrahepatic biliary atresia, IHBH: intrahepatic biliary hypoplasia
* p< 0.05 ** p<0.001

Table II. Laboratory Findings in Patients with Neonatal Cholestasis

	HCD (n=50)	EHBA (n=14)	IHBH (n=6)
·ALT (IU/L)	188±243	228±275	167±133
·GGT (IU/L)	116±94*	480±234*	321±235*
·ALP (IU/L)	143±211	870±654	679±546
· T. bilirubin (mg/dl)	7.8±4.9	14.4±5.9**	9.4±4.5
· D.bilirubin (mg/dl)	5.8±3.8	8.7±6.3	7.8±6.4

HCD: hepatocellular disease, EHBA: extrahepatic biliary atresia, IHBH: intrahepatic biliary hypoplasia
* p<0.01

found in the urine of all four patients. None of the patients were diagnosed as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD).Only one had high serum ferritin levels and high transferrin saturation. Bone marrow examinations showed storage cells in only two patients.

Abdominal ultrasonography (US) was normal in 12 cases. Thirty-three cases had hepatosplenomegaly and 25 had isolated hepatomegaly. No anatomical abnormalities were observed in the bile ducts by US. The children were also evaluated for triangular cord (TC) sign, presence and morphology of gall bladder and contraction after oral feed. The ultrasonographic findings were not significantly different between the groups (p>0.05). In twelve patients with EHBA (85.7%), three cases with IHBH (50%) and eighteen cases with HCD (36%), hepatobiliary scintigraphy (99m Tc) showed no excretion into the intestinum. Scintigraphy contributed to the diagnosis of the EHBA group in particular, more than in the other two groups (95% CI:0.35-0.58; p<0.002). Liver tissue specimens were taken from 65 patients (92.85%). Five patients with EHBA, four with CMV hepatitis, two with tyrosinemia, and two with idiopathic neonatal hepatitis had severe portal fibrosis and progression to cirrhosis. Liver tissue specimens revealed increased iron deposition in hemochromatosis and the presence of diastase-resistant hepatocyte inclusions that stain positively for periodic acid-Schiff in alpha 1-antitrypsin deficiency.

No improvement was observed in 3 of 10 patients with CMV infection who received antiviral treatment (gancyclovir). A Kasai operation was performed in 6 patients with EHBA. One of those patients who is still being followed up received a liver transplant. In addition, two patients with galactosemia, one with a hepatitis A infection, one with neonatal hepatitis associated with a congenital rubella infection, one with alpha1- antitrypsin deficiency and one with a chronic active hepatitis B infection are still outpatients being followed up by our department of pediatric gastroenterology.

Discussion

Since an early diagnosis is very important for the prognosis, the direct bilirubin level should be measured in all infants admitted with jaundice[10,11]. Mowat et al.[12], Alagille et al.[13], and Lai et al.[6] have reported that the reliability of the onset time of the jaundice was 83.3% and 57.1% in EHBA and HCD, respectively. It has been determined that herpes viruses, particulary CMV, rubella virus and probably enteroviruses may play a role in the development of intrahepatic neonatal cholestatis. Recently, it has been suggested that the reovirus type 3 and the rotavirus type 2 may be associated with EHBA[14-16]. Fischler et al.[17] showed by polymerase chain reaction (PCR) that CMV infection had a role in EHBA as well and also in intrahepatic neonatal cholestasis. CMV IgM and CMV DNA were positive in two of our twelve patients with biliary atresia. It has been reported that intrauterine CMV infection may lead to intrahepatic biliary tract hypoplasia[18] . In our study CMV Ig M and CMV DNA were found positive in one of six patients with IHBH.

Based on the period during which atresia occurs, EHBA is divided in embryonic or fetal form and a more common, perinatal form. In our study, the cases with EHBA were classified as embryonic because the jaundice started shortly after birth. Although there is frequently an association with a variety of congenital anomalies in this form of EHBA, we did not detect any anomalies in our cases.

Chang et al.[19] reported that most of their patients with neonatal hepatitis were due to a CMV infection. Fifty-six of our cases (80%) had intrahepatic cholestasis. Twenty-nine of those

(51.7%) were due to infections, ten (17.8%) to metabolic diseases, and six (10.7%) IHBH. Almost 34% of our cases with neonatal hepatitis had CMV hepatitis. CMV IgM was positive in all cases whereas CMV DNA was positive in only 12 cases. None of five infants whose mothers had positive serology for CMV IgM, had clinical or laboratory findings compatible with CMV hepatitis.

Idiopathic neonatal hepatitis has an incidence of 1:5000 births and constitutes approximately 50% of prolonged neonatal jaundice[2]. In our study, 11 (15.7%) of our cases were diagnosed as isonicotinylhydrazine (INH). This higher rate can be explained by difficulties in determining the viral factors. Jacquemin et al.[20] reported that in patients with temporary neonatal cholestasis, the jaundice resolved in 3.5 months and liver enyzmes normalized in 10 months. It has been shown that in cases with neonatal cholestasis who had a history of neonatal asphyxia, the bilirubin levels improved in 6 months and liver function tests within 1 year[21]. Similarly, we observed that in cases with intrahepatic cholestasis, the direct bilirubin levels normalized in 5.5 months, and liver enzymes within 11 months.

There are many inherited metabolic disorders which may present with hepatitis-like manifestations[22]. 14.2% of our cases were diagnosed as tyrosinemia, galactosemia, Niemann- Pick disease or alpha1-antitrypsin deficiency. Although, citrin deficiency has been reported to manifest as neonatal intrahepatic cholestasis[23], none of our patients had an abnormal amino acid analysis that demonstrated citrin deficiency. Also, none of our patients was diagnosed as having cystic fibrosis which is very common in our country due to high prevalence of parental consanguinity.

In conclusion, infectious diseases and metabolic disorders are the most common causes of neonatal cholestasis in our country because of the high rate of parental consanguinity and inadequate prenatal follow-up programs. The early identification and treatment of children with cholestasis is essential, especially of those who need corrective surgery for extrahepatic biliary atresia in order to improve long-term outcomes. It is important to keep in mind that jaundice, acholic stools and elevated GGT are better markers for patients with EHBA compared to patients with intrahepatic biliary hypoplasia and hepatocellular disease .

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