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Biliary, Anorectal and Esophageal Atresia: A New Entity ?

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DESSANTI, A., MASSARELLI, G., PIGA, M.T., PORCU, A. and DETTORI, G. *Biliary, Anorectal and Esophageal Atresia: A New Entity ?* Tohoku J. Exp. Med., 1997, **181** (1), 49-55 —— A unique case of newborn biliary atresia associated with esophageal atresia and tracheoesophageal fistula, ano-rectal atresia, Reovirus type 3 infection and an early switch of fetal into adult hemoglobin is reported. At birth, the infant, who had only one umbilical artery, was operated on by primary anastomosis of the esophagous, and descending colostomy. At six weeks of age the baby underwent a "Kasai hepatic portoenterostomy-Type I" for a EHBA Type III, Subtype C2, Subgroup O ("aplasia" of all extrahepatic biliary ducts, including the gallbladder). The absence of an artery branch for the left lobe of the liver was observed. Histologically, the liver showed a hyperplasia of the intrahepatic bile ducts due to persistence of an excess of embryologic bile ducts in "ductal plate malformation" (DPM). Specific Reovirus type 3 antibodies were found in both the mother's and baby's sera. In the postoperative period the infant developed rapid and severe liver failure and underwent a successful liver transplantation. Although in most cases EHBA appears to be a perinatal event due to a necro-inflammatory process of unknown etiology, cases associated with complex extrahepatic anomalies, may be due to different pathogenetic mechanisms supported by different causative agents operating very early in the fetal period. Viral infection seems to be the most reliable etiology. ———— biliary atresia; congenital malformations; anorectal atresia; esophageal atresia; Reovirus type 3 infection

Extrahepatic biliary atresia (EHBA) is the most common hepatic surgical disorder in infancy, representing about 50% of all neonatal cholestatic jaundice. According to Kasai's classification (Kasai et al. 1976), the main extrahepatic biliary ducts may appear as fibrous cord , fibrous mass, or may even be missing (so-called aplasia), thus producing complete obstruction of bile flow.

An early surgical approach consisting of a precise and deep dissection and

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transection of the fibrous tissue at the porta hepatis is essential to obtain appreciable clinical results, which are undoubtedly encouraging but not as good as we would wish, since the etiology of EHBA is at present unknown. The progressive destruction of well-formed extrahepatic bile ducts, due to a necro-inflammatory process, seems to be the most probable pathogenetic mechanism (Desmet and Callea 1990). In most cases, EHBA appears to be a perinatal event. However, cases associated with complex extrahepatic anomalies suggest that the causal agent may act relatively early during the organogenesis.

We report a unique case of EHBA, associated with esophageal atresia, ano-rectal atresia, vascular anomalies, early switch of the fetal into adult hemoglobin and Reovirus type 3 infection.

Case Report

A Sardinian female infant was born at 39 week gestation with a birth weight of 3,160 g. ($=50$ percentile), birth length 52.5 cm (>90 percentile), head circumference 35.5 cm ($=90$ percentile) and Apgar score 4 at 1 min, 9 at 5 min. The 37 year-old primigravida mother gave birth after 16 years of marriage. No investigation or treatment for possible infertility had been carried out. The pregnancy was uncomplicated and no drugs were taken. Physical examination of the newborn revealed a single umbilical artery, esophageal atresia with tracheoesophageal fistula and anorectal atresia (cloacal type).

Five hours after birth, the infant was operated on by transection and closure of the tracheo-esophageal fistula with primary anastomosis of the esophagous and descending colostomy with separated stoma. At that time the meconium had an unusual yellow-green colour. In the post-operative period, the baby showed an acholia fecalis and developed physiological jaundice, which turned into pathological conjugated hyperbilirubinemia in the second week of life without a jaundice-free interval, which suggested an EHBA.

The isoelectric-focusing (IEF) technique of cord blood (Masala and Manca 1991), used in routine screening of hemoglobinopathies, disclosed Hb F 49.8% (normal value; $82.1 \pm 6.8\%$), Hb A 46.2% ($17.9 \pm 6.8\%$) and Hb A_2 2%. These results were interpreted as an early switch of fetal into adult hemoglobin. Laboratory data at four weeks of life included bilirubin (T/D): 6.3/3.8 mg/100 ml, SGOT: 98 U/liter, SGPT: 62 U/liter, γGT: 1,089 U/liter, Alkaline phosphatase: 282 U/liter, Total protein: 5.3 g, Albumin: 67%, PT: 100%, aPTT: 28.7 sec. Karyotype was normal (XX, 46). Diagnostic tests for a-1 antitrypsin deficiency and cystic fibrosis were negative. The indirect immunofluorescence method, using LLC Reovirus serotype 3 infected (ATCC 231) to detect specific serum antibodies was carried out in both the infant and the mother. The infant showed specific IgM positive 1/80 and specific IgG negative. The mother, on the other hand, showed specific IgG positive 1/40 and specific IgM negative.

At six weeks of age the baby underwent a "Kasai hepatic portoenterostomy-

Type I" for an EHBA Type III, Subtype C2, Subgroup O, according to Kasai's classification (Kasai et al. 1976). In fact, all extrahepatic biliary ducts and even the gallbladder were totally missing, resulting in complete aplasia. The cholecystic bed was recognizable for the presence of a dense net of small vessels coming from the glissonian surface and from the cystic artery. The hepatic artery originated from the superior mesentheric artery and supplied blood only to the right hepatic lobe: no artery branches were visible for the left hepatic lobe.

The hepatic nerve of the ligamentum hepatoduodenale innervated only the right hepatic lobe with a single branch. The deep dissection of the porta hepatis disclosed only a small fibrous mass in the right corner.

Histologically, the liver architecture was well recognizable. Portal tracts were widened due to the presence of fibrous tissue encircling irregular and bizarre ductular structures with and without lumen but not containing bile (Fig. 1). Along the external limitant plate numerous small ducts were also visible in continuity with the liver tissue. In the portal tracts numerous small arterial vessels were present along with unconspicuous inflammatory reaction made up of few granulocytes, sparse lymphocytes and rare plasma cells. Immunohistochemical staining with cytokeratins AE1 gave an elegant demonstration of ductal plate malformation (DPM) also showing the synthesis of high molecular weight cytokeratins in liver cells near the portal tract (Fig. 2). The postoperative period was unremarkable apart from persistent jaundice associated with acholia fecalis. The infant developed rapid and severe liver failure and underwent a successful liver transplantation at the age of 5 months.

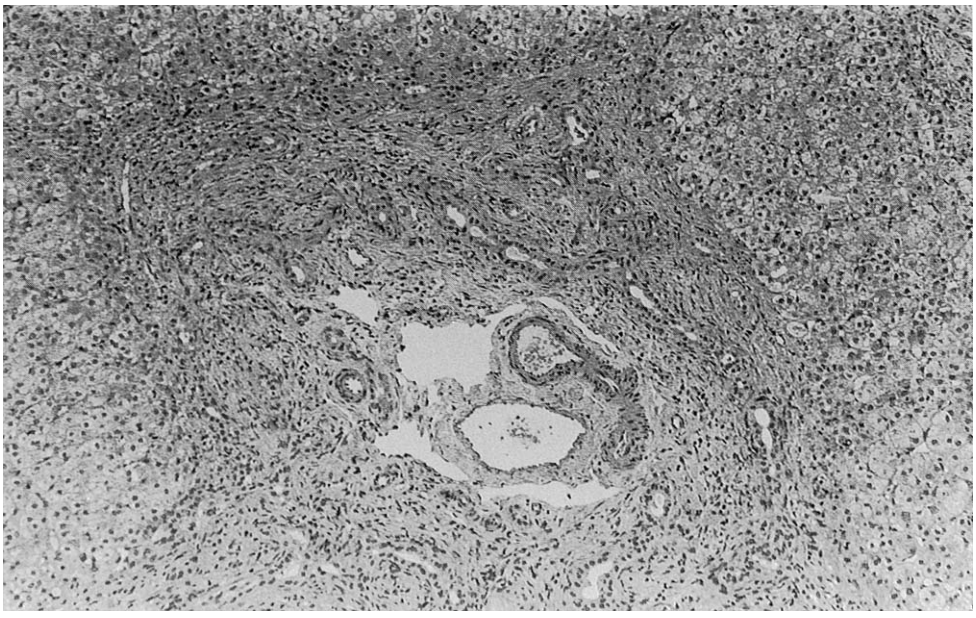

Fig. 1. Portal tracts contain irregular and bizarre ductular structures with and without lumen.

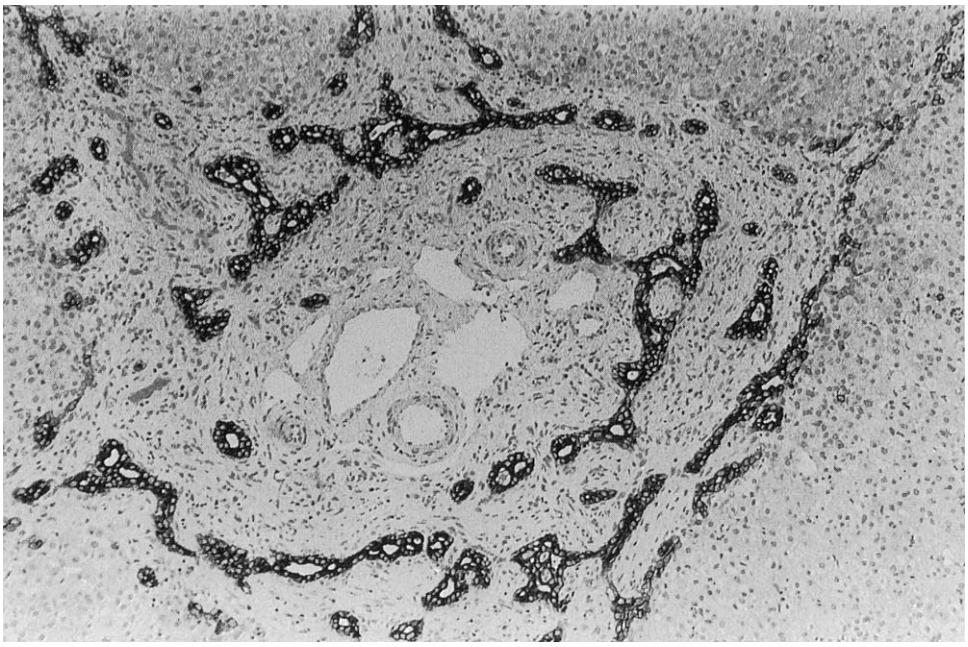

Fig. 2. Immunohistochemical staining with CK AE1 discloses the DPM and shows high molecular weight cytokeratins in limiting liver cells.

Discussion

A new era in the treatment of EHBA Type III (noncorrectable type) was successfully initiated by Prof. Morio Kasai, when in 1957 he first performed the "hepatic-portoenterostomy" operation (Kasai and Suzuki 1959). The concepts on which the Kasai operation is based are first the observation that in almost all patients with EHBA the interlobular bile ducts are patent as far as the porta hepatis during the first two months of age. Afterward they rapidly decrease and completely disappear (Chiba et al. 1976). Secondly, the fibrous mass at the porta hepatis, residual of the atretic hepatic radicles, contains numerous small biliary ducts. In the early phase of the disease these may be in conjunction with the intrahepatic bile tree (Ohi et al. 1980).

EHBA seems to be the outcome of the progressive destruction of extrahepatic bile ducts that gradually involve also the intrahepatic ones, when the bile ducts are fully formed. The pathogenesis seems to be a necro-inflammatory process of unknown etiology, starting late in the perinatal period. The histopathological features of the liver include rounding of portal tracts, edema, dilatation of lymph vessels, inflammatory infiltration and marginal ductular proliferation.

In addition to this classic form of EHBA, a second form exists in which a hyperplasia of the intrahepatic bile ducts is present in association with the typical features of EHBA (Desmet and Callea 1990). This condition may be due to a lack of remodelling or incomplete remodelling of the ductal plate, resulting in the persistence of an excess of embryologic bile ducts in DPM (Jorgensen 1977). Since DPM is frequently associated with early and extensive biliary fibrosis and

with disturbed liver architecture by the fusion of large portal areas, these cases can thus be considered as an early severe form of EHBA (Desmet and Callea 1990). In these variants, the causative process probably starts very early when the intrahepatic bile ducts are still in their embryonic shape. EHBA can occur as an isolated lesion, but in 12-20% of cases may be observed in conjunction with significant extrahepatic anomalies (Miyamoto and Kajimoto 1983; Silveira et al. 1991; Carmi et al. 1993). Polysplenia, abdominal situs inversus, intestinal malrotation, cardiovascular and/or genitourinary defects are the most frequent malformations. Isolated esophageal atresia (Chandra, 1974) and small bowel atresia (Le Coultre et al. 1983; Jolley et al. 1992) are rarely observed and anorectal atresia has been reported only once (Varty and Kapila, 1993). However, the association of EHBA with esophageal atresia and anorectal atresia, along with vascular anomalies, has not been previously reported.

In 34% of cases, EHBA is characterized clinically by cholestatic jaundice without free interval and morphologically by missing extrahepatic bile ducts. The current view is that the extrahepatic bile ducts were not formed or else became obliterated in the embryonic or fetal period of life. This condition is defined as embryonic-fetal form (Schweizer and Muller 1984).

Following the observation that Reovirus type 3 infection in weanling mice was associated with obliterative cholangitis at the porta hepatis (Bangaru et al. 1980), a significant incidence of Reovirus type 3 and/or specific antibodies was detected in EHBA patients (Sasaki et al. 1991; Morecki et al. 1982). Other studies, on the other hand, did not confirm these results (Dussaix et al. 1984; Iwami et al. 1991). Our observation of EHBA is a unique case since many of the clinical and anatomic aspects previously reported were present in the same patient. In fact, like the embryonic-fetal form, the reported case showed early cholestatic jaundice without free interval, associated with acholia fecalis preceded by a unusual yellow-green meconium. Aplasia of extrahepatic bile ducts and the occurrence of a severe form of DPM suggest that the pathogenetic agent, acting very early in the embryonic life, had influenced the remodelling of the ductal plate and inhibited the formation of extrahepatic bile ducts. The occurrence of an elevated titer of specific Reovirus type 3 antibodies both in the mother's and baby's sera, points to this virus as the causative agent of the lesions.

Chromosomal abnormalities have been reported in cases of EHBA associated with anal atresia, cardiovascular anomalies, amputation of the left leg, or polysplenia (Weichsel and Luzzatti 1965; Windmiller et al. 1965; Alpert et al. 1969; Johnson et al. 1974). Our case did not display evident chromosomal abnormalities. However, since the genes of the globin synthesis are located in chromosome 11 and 16, the occurrence of early switch from fetal to adult hemoglobin suggests a disturbance in mechanisms of genetic regulation.

In the light of our observations, EHBA has to be considered as probably due to various causes and pathogenetic mechanisms operating at different periods of

gestation, any one of which may be responsible for the different types of EHBA in relation to the time or the modality of action. Considering that liver parenchymal cells, intrahepatic bile ducts, right and left radicles and hepatic ducts derive from the pars hepatica whereas the gallbladder and common bile ducts originate from the pars cystica, the contemporary occurrence of aplasia of all extrahepatic bile ducts and the DPM could mean that the causative agent has involved both the pars cystica and pars hepatica.

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