Histopathological Studies on the Prognosis of Biliary Atresia

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CHIBA, T. Histopathological Studies on the Prognosis of Biliary Atresia. Tohoku J. exp. Med., 1977, 122 (3), 249-258 — Liver specimens from 68 cases of biliary atresia were histologically studied in reference to postoperative prognosis. The histological findings were classified into 4 to 5 groups by the degree of fibrosis, bile duct proliferation, bile plugs, giant cell transformation, and intralobular cholestasis. Furthermore, morphometrical studies of interstitial area, bile duct (ductules), intrahepatic portal vein, and intrahepatic artery were done and correlated with the age of patients and with operative results. From this study, it was shown that 1) marked fibrosis (cirrhosis) and interstitial areas occupying more than 35% of the sectional area of the liver specimen, 2) severe ductular proliferation, shown by proliferated bile ducts occupying more than 20% of interstitial areas, 3) no or slight intralobular cholestasis and 4) marked interlobular cholestasis are considered to predict poor postoperative bile excretion. —— biliary atresia; histological findings of biliary atresia

Histopathological findings of biliary atresia have been reported by many investigators and follow-up studies of long term survivors have been done to date (Miyata et al. 1974; Kasai et al. 1975). Furthermore, retrospective studies have been done on this disease (Chiba and Kasai 1975). The purpose of the present study is to correlate the histological findings of the liver with prognosis after corrective surgery for biliary atresia.

MATERIALS AND METHODS

Sixty-eight cases of biliary atresia which were operated at the Second Department of Surgery, Tohoku University Hospital for the period from 1967 through 1975 were subjected to this study.

The liver specimens were taken at the operation and were fixed in 10% formalin. After embedding in paraffin or celloidin-paraffin, several sections of 3 to 4.5 μm in thickness were cut from each block and stained with hematoxylin and eosin and Goldner's stain combined with Weigert’s stain for elastic fibers.

The following two studies were carried out on these specimens:

Bile excretion and histological findings. To investigate the relationship between histology of the liver and postoperative bile excretion, the degrees of some elementary histological changes were compared in relation to patients' age at operation and postoperative bile flow. The following 5 histological findings were used in this study: a) degree of fibrosis (cirrhosis), b) bile duct proliferation, c) bile plugs in proliferated bile ducts.

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(interlobular cholestasis), d) existence of giant cell, and e) intralobular bile stasis.

**Histometrical findings and bile excretion.** Besides histological examinations, morphometrical treatments were introduced into the present study. For this purpose, the method of "multipurpose test system" (Weibel et al. 1966) was used. This alternative coherent test system is composed of discrete short test lines of length 1 cm whose end points are arranged in a regular triangular lattice. The lattice unit is not a square, but it is an equilateral rhombus, with an angle of 60° (Fig. 1). For point counting volumetry, the end points of the test lines were used as markers. There are 400 points and 200 lines in the test system. Each specimen was transluminated and the multipurpose test system was set on the screen and the number of the end points of the test lines was counted. The points which fell on the interstitial area, bile ducts and proliferated bile ducts, intrahepatic arteries and intrahepatic portal veins were counted separately.

![Fig. 1. Test system on the photomicrograph.](image)

**Observations**

**Bile excretion and histological findings of the liver**

**Degree of fibrosis.** The degree of fibrosis was classified into 4 groups: (-) no obvious or only minimal fibrosis in the vicinity of portal areas, (+) fibrosis with narrow interlobular linking, (++) advanced fibrosis with wide definite interlobular linking with normal lobular architecture, and (+++) advanced inter- and intralobular fibrosis with regulative nodules (cirrhosis). The correlation of the degree of fibrosis with the age of patients and postoperative results is shown in Fig. 2. Fibrosis advanced with age. Cure or cure-expected cases were exclusively seen in the cases of fibrosis (-) or (+). Over 80% of the cases of poor postoperative bile excretion showed fibrosis of (+) or (++).
**Ductular proliferation.** Proliferated bile ductules usually form complex labyrinths and surrounded the interlobular bile ducts. Ductular proliferation was divided into 4 groups according to its severity: (++) marked proliferation, (++) intermediate proliferation, (+) only minimum proliferation in a few portal areas and (-) no proliferation. The correlation of severity of ductular proliferations with age at operation and operative results is shown in Fig. 3. Ductular proliferation was progressive with age. There was no case of marked ductular proliferation which showed good postoperative results.

**Interlobular cholestasis.** Bile plugs were often seen in the bile duct or dilated proliferated ductules in portal areas. Bile plug formation was classified into 4 groups: (##) bile plugs in many ductules in nearly all portal areas, (##) bile plugs in a few ductules in every portal area, (+) bile ductules with one or more bile
plugs in a few portal areas, and (−) no bile plugs in portal areas. The degree of interlobular cholestasis had a close correlation with the age of patients but did not show such a close correlation with prognosis (Fig. 4).

**Giant cell transformation.** Giant cell transformation is often seen in children with jaundice. Patients with biliary atresia had also many giant cells, but the degree of giant cell transformation was variable with their age (Fig. 5). Severity in giant cell transformation was divided into 4 degrees from numerous (+++) to none (−). There is a reverse correlation between severity of giant cell transformation and the age of patients. Marked giant cell transformation was found in many cases within 100 postnatal days but in few cases after 100 days. There was no correlation between giant cell transformation and operative results.
Fig. 6. Degree of intralobular cholestasis and age at operation. Postoperative bile excretion: ○ excellent; ● poor or none.

Intralobular cholestasis. Intralobular cholestasis was shown by bile pigmentation in liver cells and bile plugs in bile capillary. Intralobular cholestasis was classified into 3 degrees from severe (++) to none (−). It seemed that intralobular cholestasis was severer in younger patients than in older ones (Fig. 6). The prognosis of cases of no cholestasis was poor. More than a half of cured cases showed severe intralobular cholestasis.

Microscopic morphometrical findings and bile excretion

Extent of interstitial areas. The volume fraction of interstitial areas in the liver specimen was measured by the "test system" method (Fig. 7). The interstitial area was less than 50% in all cases but two. In most patients younger than the 10th postnatal week the fractions were lower than 25% but they increased with age. In almost all the cured cases, the percentage was below 30%. In cases in which the fractions were higher than 35% surgical treatment was ineffective. Fig. 8 shows the relationship between the percentage of the interstitial area and the
Fig. 8. Relationship between the percentage of the interstitial area and the degree of fibrosis. Postoperative bile excretion: ○ excellent, ● poor or none.

Degree of fibrosis. The higher the percentage of the interstitial area, the severer the degree of fibrosis.

Extent of area occupied by bile duct (ductules) and intrahepatic vessels in interstitial areas. Fig. 9 shows the relationship between the percentage of bile duct system in the interstitial area and the age at operation. The percentage of the area of bile duct and ductules in the interstitial area increased with the age of patients. All the cured cases showed the ratios under 20%. Logarithmic transformation of the ratio of the percentage of bile duct (ductules) to that of intrahepatic portal vein was made and compared with patient’s age (Fig. 10). This ratio increased with age (solid line). However, it decreased in cured cases (dotted line). The same relationship was seen between the percentage of the duct (ductules)

Fig. 9. Relationship between the percentage of bile duct proliferation and age at operation. Postoperative bile excretion: ○ excellent, ● poor or none. Solid line shows the regression line of all cases: $Y = 0.1X + 6.01$. 
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Fig. 10. Relationship between the ratio of the percentage of bile duct proliferation to the intrahepatic portal vein and age at operation. Postoperative bile excretion: ◯ excellent, ● poor or none. Solid line shows the regression line of all cases: $Y = 0.01X + 1.6$. Dotted line shows the regression line of cured cases: $Y = -0.01X + 1.89$. The ellipse shows the range of confidence at 95% level of the cured cases: $n_2^2/(70.7)^2 + n_1^2/(1.81)^2 = 1$.

Fig. 11. Relationship between the ratio of the percentage of bile duct proliferation to the intrahepatic artery and age at operation. Postoperative bile excretion: ◯ excellent, ● poor or none. Solid line shows the regression line of all cases: $Y = 0.01X + 1.1$. Dotted line shows the regression line of cured cases: $Y = -0.01X + 2.04$. The ellipse shows the confidence of 95% level of the cured cases: $n_2^2/(67.4)^2 + n_1^2/(1.63)^2 = 1$.

and that of intrahepatic artery (Fig. 11). To establish prognosis, we made references to the ellipse of the confidence range of cured cases at 95% level. Postoperative bile flow may be poor, if the ratio falls outside the ellipse.

**DISCUSSION**

A retrospective study to evaluate the effect of surgical treatment is one of the most important problems in biliary atresia. An attempt at determining
surgical indication by preoperative laboratory examinations was reported by the author (Chiba and Kasai 1975). In the present study, a further attempt was made to correlate postoperative prognosis with histological findings at the time of operation.

As to fibrosis of the liver, it is progressive with age in biliary atresia (Ohkuma 1972; Suruga et al. 1972; Kasai 1974). Postoperative bile excretion was good in patients with no or slight fibrosis. The extent of the interstitial area increased with age and it correlated well with the degree of fibrosis from this study. It had no relation with the types of extrahepatic bile duct (Shiraki 1975). Postoperative change in the degree of fibrosis was various. Saito and Ishida (1974) described that fibrosis was progressive even if postoperative bile excretion was good. However, in our experience from the follow-up studies on the histological findings, cases of successful operation in which preoperative change of the liver was not serious showed improvement of fibrosis of the liver (Kasai et al. 1975). On the other hand, in cases in which the changes of the liver were far advanced before operation, varying fibrosis still remained. Therefore, the degree of fibrosis seemed important to predict postoperative bile excretion. From this study, prognosis was poor in the cases of severe fibrosis or the cases in which interstitial area occupied over 35% of the liver specimen.

Bile duct proliferation was seen in almost all cases in varying degree. Histopathological structures of the bile duct system of biliary atresia have been studied to date (Oh-i et al. 1969; Chiba et al. 1975, 1976). Three-dimensional structures of the intrahepatic bile duct system were reported by the author with a histological reconstructive method (Chiba et al. 1975, 1976). The structure of the interlobular bile duct of biliary atresia was almost normal in the early days of life (Oh-i et al. 1969). Bile ductules had a very complex arrangement and they sometimes connected with interlobular bile ducts (Oh-i et al. 1969; Chiba et al. 1975). Bile duct proliferation was progressive with age in the first several months. However, these proliferated bile ductules would degenerate into fibrous scar in the late stage of this disease, if sufficient bile flow did not ensue upon the operation. Postoperative bile excretion was better in cases of slight bile duct proliferation than in ones of severe proliferation (Ohkuma 1972). From the present study, sufficient bile excretion could be expected in cases in which proliferated bile duct occupied less than 20% of interstitial area.

As concerns cholestasis, it is progressive with age (Ishikawa 1965). In cases of severe fibrosis, many bile plugs were found in proliferated bile ducts even in early days of life. Prognosis is poor in cases of a large number of bile plugs in the proliferated bile ducts (Ohkuma 1972). Intralobular cholestasis is often seen in early cases of biliary atresia. In the present study, prognosis is poor when there is no sign of intralobular cholestasis. Severe intralobular cholestasis is associated with slight fibrosis, and severe cholestasis (bile plugs) in proliferated bile ducts is accompanied by severe fibrosis.

Giant cell transformation is not a specific finding of biliary atresia. Many
diseases with jaundice are accompanied by giant cell transformation. In biliary atresia, it is severest 2 to 4 months after birth and gradually disappears with postnatal months (Ishikawa 1965; Shiraki 1975). Prognosis is poorer in cases without giant cell.

As for the vessels of the liver, the studies have been carried out from different view points. Intrahepatic portal vein in biliary atresia is gradually obstructed with age (Saito and Ishida 1974; Okudaira et al. 1975) and the mean number of portal vein sections in the Glisson’s sheath is inversely proportional to fibrosis of the liver (Okamoto et al. 1976). On the other hand, the number of intrahepatic arteries are increased with age in biliary atresia (Stowens 1959; Alagille 1972; Okudaira et al. 1975; Okamoto et al. 1976). The degree of fibrosis is, however, progressive with age and the vessels are compressed by fibrous tissue. In morphometrical findings, the percentage of the portal vein occupying the interstitial area is decreased with age in the present study. The same relationship is seen on the intrahepatic artery. From morphometrical study the larger the area occupied by vessels, the better the prognosis of patients of advanced postnatal months.

References


