Hepatic portoenterostomy (HPE) has made long-term survival possible for patients with biliary atresia (BA). Furthermore, several different types of hepatic masses have been described in patients with long-standing BA. These include focal nodular hyperplasia (FNH), pseudotumors and hilar nodules. Hepato-cellular carcinoma and hepatoblastoma account for the majority of primary liver cancers reported in BA patients. Cholangiocarcinoma has only been rarely reported.

This report describes the histopathologic and immunohistochemical findings of six masses that were observed among 55 explanted livers in BA patients following HPE at a referral hospital center.

**MATERIALS AND METHODS**

Eighty-one pediatric patients underwent liver transplantation (LT) for BA at Samsung Medical Center from 1997 to 2009. Of these 81 patients, 55 had previously undergone Kasai HPE. Of the 81 explanted livers, six revealed hepatic masses on routine prospective pathologic examination, and all were in patients who had previously undergone Kasai HPE.

The six explanted livers were histopathologically examined using their hematoxylin and eosin stained slides. Each case consisted of 10 to 20 slides and these slides included not only the masses, but also other parts of the explanted liver, and these slides constituted the comprehensive and representative sections for the entire specimen in each case. All the slides that made up each case were reviewed. An additional 5 µm-thick consecutive sections from five cases of formalin-fixed, paraffin-embedded hepatic lesions were cut for special staining (Masson-trichrome and reticulin staining; cases 1-5).

Immunohistochemistry was performed using a biotin-avidin peroxidase complex method on a Techmate 1000 autostainer (Dako, Glostrup, Denmark) to evaluate the relationship between the hepatic masses and the background liver. Dysplastic biliary epithelium arising from intestinal metaplasia was found in the cholangiocarcinoma. The immunohistochemical staining findings for SMA and CD34 were more prominent for the FNH-like nodules than for the cirrhotic background liver. The primary antigens used were CD34 (Dako, 1:100), smooth muscle actin (SMA) and cytokeratin 7 (Dako, 1:100). The gross photographs and medical records of these six patients were also reviewed.
RESULTS

The clinicopathologic findings of the six patients with hepatic masses are summarized in Table 1. All six had undergone Kasai HPE for BA when they were between two and six months old. After Kasai HPE, four had experienced recurrent bouts of cholangitis and two had suffered from recurrent bleeding from esophageal varices. All the hepatic masses, except the mass in case 6, were found by imaging studies conducted as part of the pre-transplantation work-up. In case 6, the mass was found incidentally by abdominal computed tomography (CT), which was performed due to an abnormal liver function test prior to dental surgery.

Abdominal CT and ultrasonography demonstrated a well-circumscribed hepatic mass in three patients (cases 1, 2, and 5) and an ill-defined mass-like lesion in the other three (cases 3, 4, and 6). In cases 1 and 2, the lesions appeared as hyperechoic or isoechoic, bulging masses that were hypervascular on ultrasonography. In case 1, the mass was enhanced during the early arterial phase and it was washed out during the delayed phase; the radiologic diagnosis was adenoma or FNH. Case 2 showed a small vascular flow signal, which entered the mass as seen on a color Doppler scan. Hepatoblastoma or hepatocellular carcinoma was suspected radiologically. In cases 3 and 4, the masses were ill-defined with peripheral enhancement and focal low attenuated areas. The radiological impression was of FNH, adenoma or a non-tumorous condition. Case 5 had a well-circumscribed low-density mass with no enhancement on abdominal CT, and this suggested hepatoblastoma, hepatocellular carcinoma or adenoma. In case 6, the mass appeared as a small vascular flow signal on color Doppler imaging.

The clinicopathologic findings of the six patients with biliary atresia after Kasai portoenterostomy are as follows:

**Table 1. Clinicopathologic findings of hepatic masses in 6 patients with biliary atresia after Kasai portoenterostomy**

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Serum AFP (ng/mL)</th>
<th>Age at HPE (mo)</th>
<th>Complication after Kasai</th>
<th>Age at mass detection</th>
<th>Age at LT</th>
<th>Pathologic diagnosis</th>
<th>Location</th>
<th>Lesion (size, cm)</th>
<th>Liver weight (g)</th>
<th>Other findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>WNL</td>
<td>1 yr 5 mo</td>
<td>Cholangitis, HSM</td>
<td>1 yr 6 mo</td>
<td>FNH-like nodule, Right lobe, anterior seg</td>
<td>Well circumscribed mass with fibrous septa (2.7)</td>
<td>520</td>
<td>Biliary cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>WNL</td>
<td>1 yr 5 mo</td>
<td>Cholangitis, PH, HSM</td>
<td>1 yr 6 mo</td>
<td>FNH-like nodule, Left lobe, medial seg</td>
<td>Well circumscribed mass with fibrous septa (3.2)</td>
<td>480</td>
<td>Biliary cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>WNL</td>
<td>2 yr 4 mo</td>
<td>Cholangitis, HSM</td>
<td>3 yr</td>
<td>Left lobe (S4)</td>
<td>Ill-defined mass-like lesion (7)</td>
<td>540</td>
<td>Biliary cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>WNL</td>
<td>4 yr 3 mo</td>
<td>Esophageal varix, PH</td>
<td>4 yr 5 mo</td>
<td>Left lobe (S4)</td>
<td>Well circumscribed mass (2.3)</td>
<td>545</td>
<td>Biliary cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>17,540</td>
<td>4 mo</td>
<td>Cholangitis, PH, HSM</td>
<td>7 mo</td>
<td>Mesenchymal hamartoma, Right lobe (S8)</td>
<td>Well circumscribed mass (2.5)</td>
<td>336</td>
<td>Biliary cirrhosis, DPM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>WNL</td>
<td>13 yr 3 mo</td>
<td>Esophageal varix, PH, spleno-megaly</td>
<td>13 yr 4 mo</td>
<td>Intrahepatic ducts of right lobe</td>
<td>Papillary mass (3)</td>
<td>1,203</td>
<td>Biliary cirrhosis, hepatic stones</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AFP, alpha-fetoprotein; HPE, hepatic portoenterostomy; LT, liver transplantation; F, female; M, male; WNL, within normal limits; HSM, hepatospleno-megaly; FNH, focal nodular hyperplasia; PH, portal hypertension; LRN, large regenerative nodule; DPM, ductal plate malformation.

Chiari syndrome based on the radiologic findings. None of the patients had heterotaxy, which was confirmed by all the radiologic examinations.

The time between detection of the mass and Kasai HPE was two months for case 3, 14-15 months for cases 1 and 2, two to three years for cases 3 and 4, and 13 years for case 6. Prior to LT, the levels of serum alpha-fetoprotein and carcinoembryonic antigen were within the normal limits in all the patients except case 5. Case 5 had an elevated alpha-fetoprotein level of 17,540 ng/mL. Unfortunately, case 6 died due to multiple metastases from cholangiocarcinoma to the transplanted liver one year after LT. The other five patients were still alive at the time of writing this report (mean follow-up, 56.6 months post-LT; range, 22 to 109 months).

**Pathologic characteristics**

**Gross findings**

The explanted livers weighed from 336 to 1,203 g and they showed marked cirrhotic changes with small and dark greenish parenchymal nodules, with or without multiple dilated bile ducts filled with bilirubin casts. There were multiple black stones in the dilated intrahepatic ducts of case 6 and the stones measured up to 1.3×1 cm.

Gross examination of the livers from cases 1 to 5 revealed well-circumscribed hepatic masses that measured from 1.2×1.0 to 5.5×4.0 cm (mean, 3.5 cm) (Fig. 1A-E). The colors and textures of the masses were similar to the surrounding cirrhotic livers, although they were more bile stained except for case 5. In case 5, the lesion was paler than the surrounding liver and it...
was well encapsulated. Intervening fibrous septa were observed within the masses in cases 1 to 5. Case 6 showed a pinkish gray papillary mass filling the right intrahepatic duct at the S8 segment (Fig. 1F).

**Fig. 1.** Gross photographs of the hepatic masses in the explanted livers from 6 patients with biliary atresia after Kasai hepatic portoenterostomy. (A) Case 1, focal nodular hyperplasia (FNH)-like nodule. (B) Case 2, FNH-like nodule (arrows). (C) Case 3, large regenerative nodules (LRN, arrowheads). (D) Case 4, LRN (arrows). (E) Case 5, mesenchymal hamartoma. (F) Case 6, cholangiocarcinoma (arrowheads).

**Histologic findings**

Histologically, all six explanted livers revealed only fibrotic connective tissue with or without small bile ducts at the hepatic bed and hilar regions, which was all compatible with BA. The livers also showed micronodular cirrhotic changes with small...
liver cell nodules surrounded by thick fibrous septa. This fibrous septa showed lymphocytic infiltration, marginal ductular proliferation, prominent arterial branches and attenuated portal vein branches. Bile lakes and abscess formation were observed in cases 1, 2, and 6, and giant cell transformation of hepatocytes was observed in case 5. Cholestasis, feathery degeneration, bile plugs filling the bile ducts and glandular arrangements of hepatocytes occurred to various degrees in all the cases. These histological findings of the 6 livers were consistent with secondary biliary cirrhosis due to extrahepatic BA.

The masses of cases 1 and 2 had similar histologic features. Both were subdivided into smaller hepatocellular nodules by fibrous septa and fibrous scar-like tissues that contained lymphohistiocytes and bile ductules. Masson-trichrome staining in these two cases more vividly revealed the fibrotic components of the masses and the background cirrhotic liver. The fibrous septal tissue was paler (meaning it was later-formed immature collagen deposition) in the masses than in the background by Masson-trichrome staining (Fig. 2A, B). The radiating central scars were not distinctive in these cases, and the fibrous septa consisted of vascular connective tissue that contained many small arteries and scant bile ductules (Fig. 2C, D). The portal vein and large arteries with eccentrically thickened muscular walls were rarely observed in the fibrous septa. The hepatocytes of the masses were arranged in two-cell thick plates separated by sinusoidal spaces lined by a single layer of endothelial-like cells. The liver cells resembled those of normal liver, but they were slightly larger and paler. Cholestatic rosettes, canaliculal cholestasis and bile pigment phagocytosis by Kupffer cells were also observed in the masses. The pathologic features of chronic cholestasis were more severe in the masses than in the cirrhotic liver parenchyma. The pathologic findings of cases 1 and 2 were compatible with FNH-like nodules.

The histopathologies of the masses were similar in cases 3 and 4. At low power magnification, the lesions were not well delineated in focal areas because the hepatic lobules between the lesions and the cirrhotic parenchyma were continuous (Fig. 2E). The lesions consisted of large lobules of hepatic parenchymal tissue incompletely interrupted by a few thin fibrous septa. Within the lesions, the septa were continuous with fibrous septa surrounding cirrhotic nodules in some areas. The liver cells were arranged in one- or two-cell plates with intervening sinusoidal spaces (Fig. 2F). The cholestasis within the masses was similar to that in the cirrhotic livers. These findings were compatible with macrogenenerative nodules or large regenerative nodules (LRN).

Case 5 demonstrated a well-encapsulated solid mass, which consisted of a disorganized proliferation of primitive mesenchyme, dysmorphic bile ducts and hepatic parenchyma (Fig. 2G). The dysmorphic bile ducts showed frequent ductal plate malformation and hepatocyte-bile duct transition. The hepatocytic component that formed cords, islands or lobules of multiple cell thickness with sinusoidal spaces showed severe giant cell formation, ballooning degeneration and occasionally rosette formation with bile pigments or concretions. The hepatocytic changes were more severe in the lesion than in the cirrhotic liver. In particular, multifocal extramedullary hematopoiesis was observed at the sinusoids in the mass. These histologic findings are consistent with solid mesenchymal hamartoma (MH).

Case 6 demonstrated papillary adenocarcinoma associated with hepatolithiasis in the large intrahepatic bile duct. The tumor showed mainly intraductal papillary growth with intraluminal spread along the dilated intrahepatic ducts, but focal parenchymal extension and frequent endolymphatic tumor emboli were also identified (Fig. 2H). In the tumor vicinity, intestinal metaplasia was identified in the adjacent hyperplastic and dysplastic bile duct epithelium. Some bile ducts contained stones, and the mural glands of the bile ducts showed hyperplastic changes secondary to stones.

**Immunohistochemical findings**

The cirrhotic livers and FNH-like nodules contained numerous SMA-positive cells in fibrous septa. In addition, SMA was diffusely positive in the perisinusoidal cells of both FNH-like nodules, and this was in contrast to the focal SMA expression in the perisinusoidal cells of the cirrhotic nodules (Fig. 3A, B). However, the LRNs showed a pattern of SMA expression in the perisinusoidal cells similar to that of the cirrhotic nodules; this was rare in case 3 and diffuse but strong in case 4 (Fig. 3C, D). MH showed a strong SMA expression in the primitive mesenchymal component (Fig. 3E) and MH showed a multifocal SMA expression in the hepatocytic component within the mass and in the cirrhotic nodules.

Sinusoidal capillarization of the hepatic masses was evaluated by immunostaining for CD34. All the cases showed a little CD34 staining along the marginal sinusoids in the cirrhotic nodules (Fig. 3F). This immunoreactive pattern was also observed in both LRNs, but the FNH-like nodules showed more widespread CD34 immunoreactivity with a more pronounced inflow pattern extending to the near centrilocellular area than that in the background cirrhosis (Fig. 3G).

CK7 stained the normal and abnormally proliferating bile
duct epithelium throughout the livers. The CK7 stained slides revealed the paucity of interlobular bile ducts compared with the distribution of interlobular bile ducts of the background cirrhotic nodules in cases 1 and 2 (Fig. 3H). On the side of the hepatocytes, in both cirrhotic and hepatic masses, the hepatocyte staining for CK7 was minimal in cases 1, 3 and 5, as well as

Fig. 2. Histologic findings of the hepatic masses. (A, B) A relatively well-defined focal nodular hyperplasia-like nodule with a thick fibrous capsule and fibrous scar-like tissue septa dividing the mass. Note the different collagen maturing patterns between the background cirrhosis and the mass (A, case 1, M-T stain; B, case 2, M-T stain). (C) The fibrous septa consist of vascular connective tissue that contains many small arteries and scant bile ductules (case 1). (D) A liver cell cord with two-cell thickness (case 1). (E) A large regenerative nodule with an inconspicuous border continuing to the cirrhotic fibrous septa (case 3, M-T stain). (F) The liver cells are arranged in one- or two-cell plates with intervening sinusoidal spaces (case 4). (Continued to the next page)
in case 2. In particular, case 4 showed diffuse strong hepatocyte staining in both the cirrhotic background and the masses (Fig. 3I, J). The positively stained cells were scattered singly or in small aggregates.

**DISCUSSION**

FNH usually occurs in the non-cirrhotic liver. According to the International Working Party, FNH is defined as a mass composed of benign appearing hepatocytes in a liver that is otherwise histologically normal or nearly normal. However, several studies have described a type of focal lesion in cirrhotic livers that is morphologically similar to classical FNH. This new type of lesion has been described as an “FNH-like nodule” and it has been associated with chronic liver diseases such as cryptogenic liver disease, viral hepatitis B and C, primary biliary cirrhosis and alcoholic cirrhosis. FNH has rarely been described in BA patients, with only three case reports in the literature. These reported cases are also referred to as FNH-like nodules because they reported cirrhosis in the background liver.

In this study, the FNH-like nodules showed variable degrees of fibrotic scarring. Diffuse immunoreactivity for SMA was observed in the perisinusoidal cells of all the FNH-like nodules, whereas no such expression was observed throughout the background livers. A finding of SMA positive myofibroblast-like cells among liver mesenchymal cells is considered to be closely related to variable hepatocellular damage, such as that of hepatic fibrosis. Thus, myofibroblast-like cell activity in the FNH-like nodules of our cases appears to be related to diverse degrees of fibrotic change. Furthermore, myofibroblast-like cells seem to increase the fibrotic nature of the masses with time.

The hepatic masses in cases 3 and 4 could be defined as LRNs, which are multi-acinar regenerative nodules with diameters larger than 5 mm. Ijiri et al. reported on patients with hilar nodules diagnosed as LRNs after portoenterostomy, and some of the cases had a much better prognosis. In our cases, the LRNs showed a myofibroblast-like cell distribution, which was similar to that of the background liver and different from that of the FNH-like nodules. This observation suggests that LRNs have preserved liver structure, which suggests a more favorable prognosis. Furthermore, this finding could be a clue for making the differential diagnosis of FNH-like nodules and LRNs.

FNH-like nodules and LRNs are believed to represent portal tract anomalies. In our cases, we hypothesized that the vascular changes induced by portoenterostomy and the consequent blood flow alterations induced the development of the FNH-like nodules or LRNs. In this study, the time that elapsed between portoenterostomy and the detection of the FNH-like nodules was an average of one year, which is substantially less than the five to nine years previously reported. In view of the fact that the sizes of FNH rarely change, it is believed that FNH-like nodules can be induced relatively soon after portoenterostomy and that the early vascular change differences are responsible for the sizes of the FNH-like nodules.

CD34 is an endothelial surface adhesion molecule that is normally expressed in the portal and central veins of the liver, but not in liver sinusoids. However, when the vascular perfusion is altered, the sinusoidal endothelium undergoes phenotypic alterations, and CD34 may then be expressed along the sinusoids in the inflow areas. In this study, all the lesion specimens showed strong CD34 staining along the periportal sinusoids.

![Fig. 2.](image)

(Continued from the previous page) (G) Mesenchymal hamartoma showing the disorganized proliferation of primitive mesenchyme and dysmorphic bile ducts (case 5). (H) Cholangiocarcinoma with invasive growth into the adjacent stroma (case 6). M-T, Masson-trichrome.
that had undergone fibrosis, which reflects altered blood flow. Furthermore, a remarkable CD34 expression was observed in the FNH-like nodules, which suggests that the pathogenesis of FNH-like nodules is more closely associated with blood flow than that of the LRNs.

CK7 immunostaining was performed to emphasize that the
patterns and density of the bile ducts inside and outside the main lesions. It helps distinguish FNH-like nodules from the background parenchyma. Hepatocytes do not express CK7 in the normal liver, but an aberrant hepatocyte CK7 expression has been described in pediatric cholestatic diseases, including extrahepatic BA. Although a previous report found that CK7 is useful for distinguishing normal liver parenchyma from FNH, in this study, the CK7 staining patterns in the hepatic masses were similar to those in the background liver.

Hepatic MH is an uncommon benign tumor, and it has been reported to be associated with BA in two patients (including our case). MH is usually cystic and MH is mainly composed of myxoid mesenchymal tissue with tortuous or cystic bile ducts, but the solid form has different clinicopathologic features from cystic MH. One of us (YLS) studied and reported that the solid form has a higher serum level of alpha-fetoprotein, smaller bile ducts and more frequent proliferation of vessels. The serum alpha-fetoprotein level was related to the amount of hepatocytes. Our case consisted of fibromyomatous mesenchymal tissue that contained branching bile ducts similar to malformed ductal plates and hepatic lobules, which belonged to a solid type of MH.

The etiology of MH is not well known, but ischemia due to an anomalous vascular supply and abnormal placental development have been suggested. The occurrence of MH in this study can also be explained by the vascular changes induced by HPE, like FNH or LRN. Cytogenetic abnormalities have recently been found to be associated with MH, but the significance of an abnormal karyotype is still unclear in terms of the pathogenesis and diagnosis of MH. The pathogenesis of the acquired MH in the present study might have been associated with the postnatal change of HPE, rather than with a congenital cytogenetic alteration, because it was not found until the work-up for LT. However, the previously reported case of MH was found...
during a diagnostic laparotomy for icterus, and a surgical procedure to correct the BA was performed after the removal of the MH.\textsuperscript{18} The clinical presentation and the pathologic processes of the two cases are different from one another. In other words, it is still difficult to explain the pathologic correlation between MH and BA, which are both unusual diseases.

Cholangiocarcinoma is the second most common type of hepatic carcinoma worldwide and in South Korea, but it is extremely rare in pediatric patients, and only a handful of cases associated with biliary atresia\textsuperscript{9} or congenital biliary dilatation\textsuperscript{22-23} have been reported. The risk factors of cholangiocarcinoma include primary sclerosing cholangitis, liver fluke infestation, congenital fibropolycystic liver, bile duct adenoma, biliary papillomatosis, choledochal cysts, hepatolithiasis, obesity, Thorotrast, chronic viral hepatitis, cirrhosis, chronic non-alcoholic liver disease and chemical carcinogens, such as nitrosamines.\textsuperscript{24-26} Further, some cases of cholangiocarcinoma after surgery for biliary-enteric drainage have been reported.\textsuperscript{27-29} Given these etiologies, the metaplasia-dysplasia-carcinoma sequence provides a possible explanation for cholangiocarcinoma formation. Our cholangiocarcinoma case is also considered to provide evidence of the metaplasia-dysplasia-carcinoma sequence via the intestinal metaplasia in the stone-containing intrahepatic bile ducts.

There are no previous studies regarding the prevalence of masses in patients with BA, and there are only sporadic reports about the types of masses found in livers with BA. In the present study, all the masses were reviewed in one referral center for a certain period. Uncommon benign and malignant tumors were found in the patients who underwent LT after portoenterostomy for BA. Furthermore, this frequency of occurrence was much higher than is usually observed (six of 55 patients, 11%). This may be due to the fact that these patients had many reasons to undergo radiologic evaluation.

However, Kasai HPE and its consequences could act as risk factors or as contributory factors to the development of liver masses. It is well known that Kasai HPE can induce hemodynamic changes, and the benign lesions such as FNH-like nodules, LRN, and MHs are believed to develop from an abnormal vascular supply. However, regarding cholangiocarcinoma, cholangitis due to BA per se, rather than due to Kasai HPE, could be the cause of the malignancy. Hence, in our case 6, hepatolithiasis could have been a contributory factor.

Based on this report, all of the lesion types encountered in this series should be included in the differential diagnosis of a newly formed hepatic mass in BA patients after portoenterostomy. No previous report has been issued describing the malignant evolution of FNH-like nodules or LRNs, which indicates that small FNH-like nodules or LRNs can be followed-up without intervention. However, biopsy is required in the cases where malignancy due to another type of tumor is suspected. Furthermore, as shown by our case 5, an elevated alpha-fetoprotein level (\textgreater{} 10,000 ng/mL) cannot be used alone to differentiate hepatocellular carcinoma in BA patients.

REFERENCES


