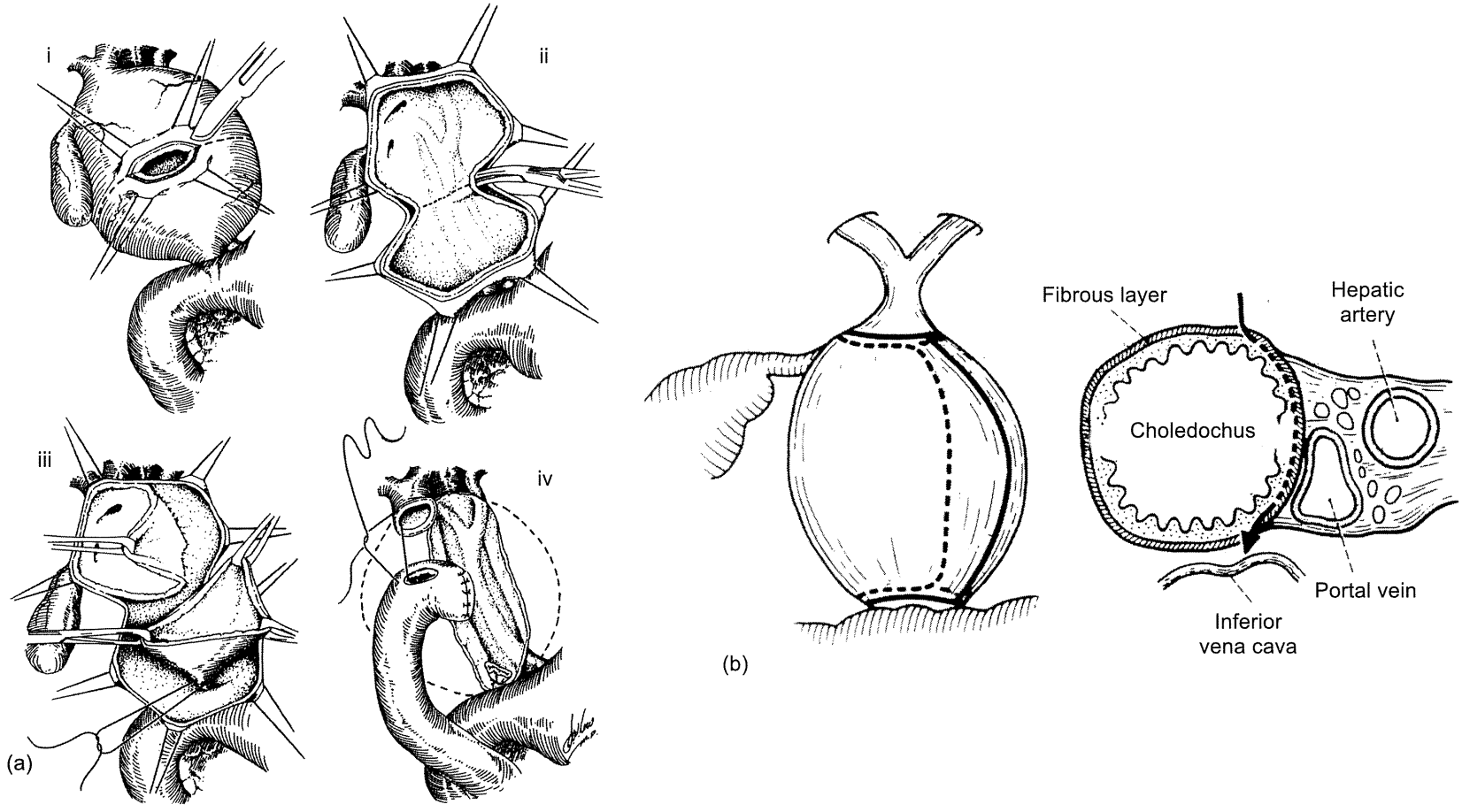

Figure 8. (a) Lilly's method: The cyst wall is incised transversely and a plane of dissection is selected in the posterior wall which separate into a thin outer and thick inner layer. After completing the separation, the inner wall is divided, completing a circumferential transverse incision. The inner cystic wall stripped from the outer cystic wall by blunt dissection except for the thin shell of posterior wall still adherent to the portal vein and hepatic artery; (b) Okada's method: Shallow longitudinal incision is made in the dilated bile duct close to its left edge with the help of a sharp scalpel. The dissection is terminated as it is carried well into the posterior aspect of the ductal wall.

divided, completing a circumferential transverse incision. The inner cystic wall is stripped from the outer wall by blunt dissection except for the thin shell of the posterior wall still adherent to the portal vein and hepatic artery. In contrast in Okada's method a sharp scalpel is used to make a shallow longitudinal incision in the dilated bile duct as close to its left edge as possible.[48] Longitudinal incision is preferable to proceed with the dissection in a superficial layer just below the serosa. The dissection is terminated as it is carried well into the posterior aspect of the ductal wall. The advantage of this approach is avoidance of injury of the vascular networks in the hepatoduodenal ligament because the hepatic artery and portal vein are left undisturbed. However, this approach causes massive bleeding because there are many vessels in this plane.

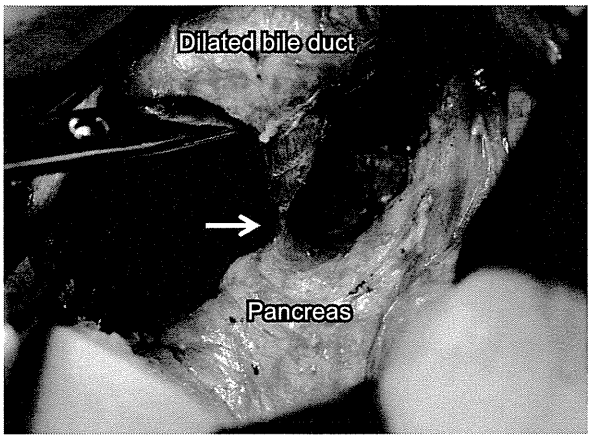

Figure 9. Exteriorization of the narrow distal segment (arrow). The narrow distal segment connecting the cyst and the main pancreatic duct is located at the lower portion of the cyst.

Further dissection reveals that the narrow distal segment connecting the cyst and the main pancreatic duct is located at the lower portion of the cyst (Figure 9). Careful surgery is required to avoid damaging the main pancreatic duct. The pancreatic portion of the common bile duct should be divided at a level just proximal to the junction with the pancreatic duct. Repeated radiographic examinations with radiopaque clips placed on the line of division are helpful for determining proximity to the junction[49] (Figure 10).

For patients with protein plugs stuck in the common channel, irrigation with saline solution through a thin tube placed in the common channel or removal using a blunt spoon through the narrow segment is recommended, or intraoperative cyst endoscopy with a pediatric cystoscope may be useful to irrigate the common channel and wash out any debris or protein plugs[46,50] (Figure 11). Incomplete excision of the cyst in the pancreas causes retention of pancreatic juice and secretes mucin from its developed periductal glands and it may be related closely to the formation of protein plugs and pancreatic stones or carcinoma

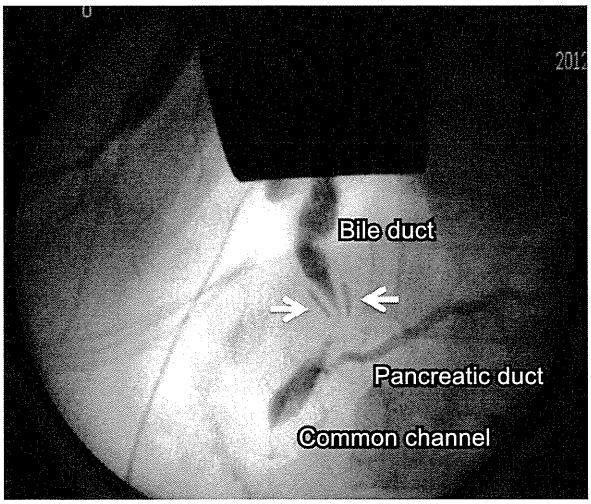

Figure 10. Radiographic examination with radiopaque clips (arrows). Radiographic examinations with radiopaque clips are helpful for determining proximity to the pancreaticobiliary junction.

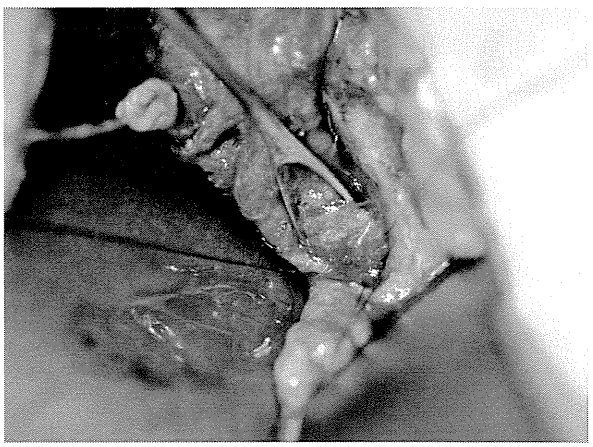

Figure 11. Protein plugs. Removal of protein plug with the help of a surgical spoon.

as a late complication.[51] The distal narrow segment is ligated carefully with an absorbable suture to prevent narrowing of the pancreatic duct. Complete cyst excision is recommended to prevent the development of chronic or recurrent pancreatitis after surgery. Meanwhile, surgical sphincteroplasty was ineffective in preventing the recurrent protein plug formation in the residual duct.

Next, the cyst is mobilized proximal to the common hepatic duct. The hepatic duct near the bifurcation is transversely incised for confirmation of the stenoses at the orifice of the left and right hepatic ducts. Primary strictures of the hepatic ducts near the hilum and intrahepatic ducts are frequently seen in patients with Todani's type IV-A.[52] There are two different types of stenosis—membranous and septal[52] (Figure 12). Membranous stenosis is characterized

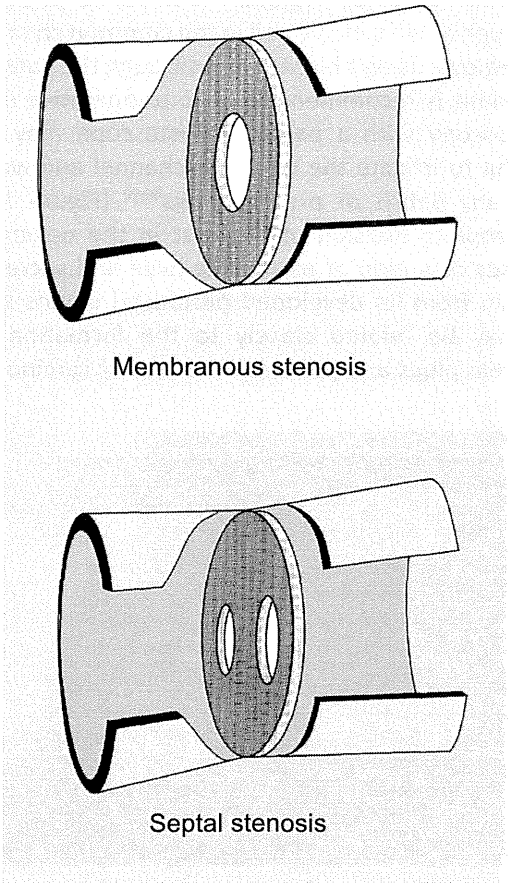

Figure 12. Two different types of the stenosis—membranous and septal stenosis.

Figure 13. Membranous stenosis. It mostly exists close to the hepatic hilum and is about <2 mm thick.

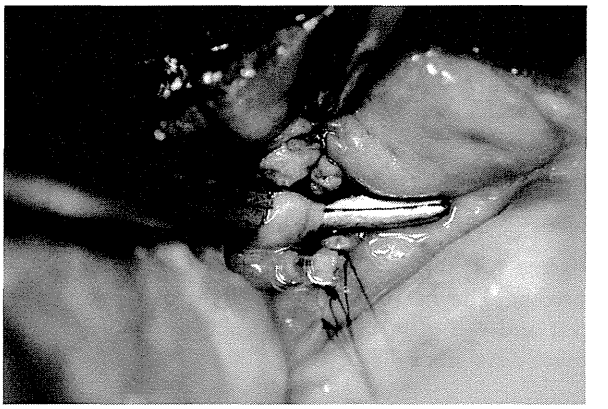

Figure 14. Septal stenosis. It mostly exists close to the hepatic hilum, and it continues upto the bile duct wall like a cord.

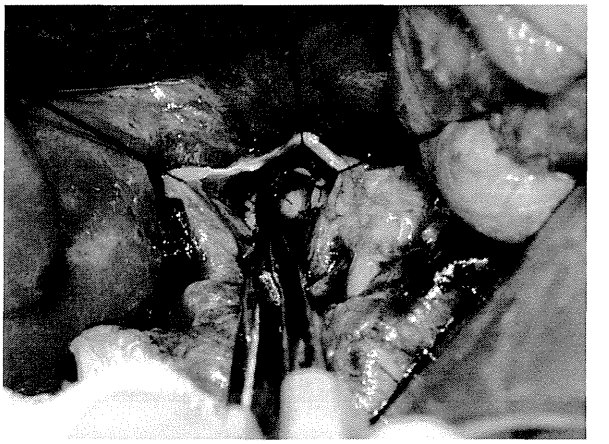

Figure 15. Hepatic hilum after resection of the stenoses from inside the lumen of the common hepatic duct.

by the presence of a thin wall (<2 mm) with a narrow orifice (Figure 13). Septal stenosis is characterized by a slender column of tissue, which divides the duct into two narrow lumens (Figure 14). Histologic examination of these two types of stenoses showed no difference with the cyst wall.[52] When membranous or septal stenoses are found around the hepatic hilum, they should be excised from the divided end of the common hepatic duct at the hepatic hilum[53] (Figure 15). Alternatively, stenoses are corrected by extending the incisions along the lateral walls of the hepatic ducts to obtain a large anastomosis.[54,55] Wide anastomoses and smooth bile flow are essential to prevent the complication of ascending cholangitis particularly for Todani's type IV cysts.[56] For patients whose stenosis is located distant from the hepatic hilum and cannot be reached from there endoscopic resection of the stenosis or hepatectomy may be indicated. If no stenosis is present at the bile ducts, the cyst is transected at the common hepatic duct.

The most common reconstructive procedures are reconstruction by a Roux-en-Y hepaticojejunostomy. Hepaticoduodenostomy reconstruction has advantages of relative simplicity, particularly with the laparoscopic approach, postoperative endoscopic accessibility of the anastomosis, and restoration of relatively physiologic bile drainage.[57] However, hepaticoduodenostomy reconstruction is more frequently complicated by bile gastritis, cholangitis, and is associated with a higher ongoing risk of cholangiocarcinoma.[58,59]

Treatment Policy for *Forme Fruste* Cysts

There is general agreement that the dilated common bile duct should be resected in view of the high incidence of postoperative complications such as biliary stones and an increased risk of malignancy of the bile duct in later life. On the other hand, the treatment for *forme fruste* or pancreaticobiliary maljunctions without biliary dilatation in adults remains controversial. Simple cholecystectomy in adults presenting with *forme fruste* has been justified because of the high incidence of associated gallbladder carcinoma. In other words, in adults, excision of the common bile duct was regarded as unnecessary because bile duct carcinomas develop rarely. However, Todani et al[60] collected and analyzed 1,062 cases of pancreaticobiliary maljunction from the Japanese literature and found the incidence of carcinoma of the biliary duct in *forme fruste* to be as high as 12.2%. Although the mechanism of carcinogenesis in pancreaticobiliary maljunctions is not yet clearly understood, regurgitation of pancreatic secretions may play an important role in provoking malignant changes.

Given the long lifespan of pediatric patients, pancreaticobiliary maljunction must be treated. Therefore, a simple cholecystectomy as prescribed for adults is not justified in children, because pancreatic secretions continue to come in contact with the bile duct because of pancreaticobiliary maljunction even after simple cholecystectomy.[61–63] The treatment of choice for children is extrahepatic bile duct excision with a Roux-en-Y hepaticojejunostomy.[13,49] However, close postoperative follow-up is required because postoperative stricture formation and recurrent cholangitis are problems associated with reconstruction of the nondilated biliary tree.[13]

Laparoscopic Procedures for Choledochal Cyst

Laparoscopic surgical excision of choledochal cyst is a feasible surgical alternative. Laparoscopic surgery for choledochal cysts was first performed on a 6-year-old female child by Farello et al.[64] Subsequently, many authors reported the safety and feasibility of the procedure in children. With the magnified laparoscopic view, dissection of the cyst wall is clearer than in open surgery. It is possible to achieve surgical removal of a choledochal cyst and Roux-en-Y reconstruction with the minimal access approach. Regarding the operation for choledochal cyst, it is extremely important to investigate preoperatively the entire pancreaticobiliary duct system thoroughly, especially with respect to the presence of a stricture of the intrahepatic bile duct and protein plugs in the common channel.[65] However, the proper management of the distal part of the cyst from the head of the pancreas and dissection of the stenoses of the hepatic hilum are the most difficult parts of the entire laparoscopic

procedure. The main limitation to completing the procedure laparoscopically is the presence of adhesions, which form as a result of recurrent cholangitis or pancreatitis.[66] However, the advantages of the laparoscopic procedure are: excellent intraoperative visualization, no early postoperative pain, no laparocele, prevention of adhesions, rapid resumption of peristalsis, excellent esthetics, and quicker resumption of activities.[66] When these reasons are taken into consideration, laparoscopic surgery may be a good method cosmetically if limited to a large cyst of Todani type I case without biliary stenosis in the hepatic hilum and no protein plugs in the common channel.

Management for the Patients with Prenatal Diagnosis

There are some reports that operative treatment of choledochal cyst in early infancy is both safe and effective, and moreover, may prevent serious complications later in life. However, the correct timing of surgery for antenatally-diagnosed yet asymptomatic choledochal cysts is not defined and whether surgery should be performed during the neonatal period is still controversial.[67,68] The indications for surgical cyst excision or only biliary drainage operation are persisting jaundice, abnormal liver function tests, and increasing cyst size. Neonatal surgery on a thin-walled choledochal cyst can be technically difficult and may injure surrounding structures such as the hepatic artery, portal vein, or pancreatic duct, and cause further anastomotic complications such as leakage or stricture.[67] Therefore, asymptomatic choledochal cyst patients are monitored closely for 2–3 months through ultrasonography and liver function tests. If during this period the patient is examined with findings of intrahepatic bile duct stricture and the pancreaticobiliary maljunction he must undergo radical operation.

Management for Spontaneous Perforation of the Bile Duct

Surgical recommendations for the spontaneous perforation of the bile duct include simple peritoneal drainage, operative closure of the perforation, T-tube drainage or primary cyst excision. The patient should be in a stable condition necessary for a long operation. A cholangiogram should be performed to detect the protein plugs and pancreaticobiliary maljunction and the presence of the stenoses of the hepatic duct. Therefore, the first step should be treated primarily by external biliary drainage e.g., T-tube.[37] After improvement of the general condition of the patient and washing out the protein plugs in the common channel, complete resection of the choledochal cyst should be performed.

Indication for Surgery in Asymptomatic Cases

Asymptomatic cases were reported to account for 26% of all adult patients regardless of bile duct dilatation, and this percentage increased to 33% when considering patients of at least over 40 years of age.[11] The problem is that complicating lesions, such as biliary tract cancer, were detected in 62.5% of asymptomatic adults over 40 years of age. In addition, patients with forme fruste or pancreaticobiliary maljunction without bile duct dilatation, for the most part, have only a few minor symptoms or are without symptoms before carcinoma occurs, and gallbladder carcinoma is usually detected late, at an unresectable stage. Therefore, surgical treatment is required once pancreaticobiliary maljunction either with or without bile duct dilatation is diagnosed irrespective of the presence or absence of symptoms.

POSTOPERATIVE COMPLICATIONS

Cholangitis and Hepatolithiasis

Cholangitis, secondary to anastomotic stricture is more frequent in patients who have undergone operations with anastomoses distal to the bifurcation of the hepatic duct and in those with Todani's type IV-A.[56] On the other hand, biliary reconstruction followed by cyst excision neutralizes the defense mechanism of Oddi's sphincter against bacteria, and causes bile infection. Biliary reconstruction with a Roux-en-Y loop has been thought to be relatively free from reflux of small bowel contents but may not

prevent the countercurrent of the bacteria. A combination of ductal stenoses and bile infection with gram-negative enterobacteria through a bilioenterostomy may play an important role in recurrent ascending cholangitis and intrahepatic calculus formation after excision of a choledochal cyst. However, as for the main cause of the intrahepatic calculi formation following excision of the choledochal cyst, there is a report that it is not anastomotic strictures but the congenital stenoses of the intrahepatic bile ducts.[53]

Intrahepatic calculi form at a rate of 2.7–10.7% after cyst excision.[54,69] Recurrent ascending cholangitis and intrahepatic stone formation need reoperation. Therefore, a wide anastomosis, resection of the primary ductal stenoses and smooth bile flow should be attained after the initial operation.[53,56]

Pancreatic Duct Injury

When the pancreatic duct is injured at operation, pair the duct with 6-0 absorbable sutures threads or put a thin tube into the damaged pancreatic duct from the pancreatic duct to duodenum. Alternatively, endoscopic retrograde pancreatic drainage tube should be inserted when pancreatic duct injury is noticed by a large quantity of pancreatic juice flowing through the drain postoperatively.[70]

Carcinoma

Cyst excision has been recognized as the definitive operation for choledochal cyst; however, reports of bile duct cancer after cyst excision during a long-term follow-up are gradually increasing. Thistlethwaite and Horwitz[71] reported that cholangiocarcinoma occurred on the anastomotic portion 4 years after cyst excision. Moreover, Gallagher et al[72] reported cholangiocarcinoma developed in the intrahepatic cysts although the choledochal cyst had been excised 7 years previously. Watanabe et al[40] collected 23 patients reported to have bile duct cancer after cyst excision. The incidence of bile duct carcinoma is still extremely high even after excision of the extrahepatic bile ducts in choledochal cysts; it is reported to be 0.7–5.4%, and the relative risk of developing cholangiocarcinoma is not thought to decrease despite performing biliary diversion.[40]

A direct connection between the intestinal tract and biliary tree, and reflux of activated pancreatic juice and intestinal bacteria into the biliary tract are considered to be the factors causing chronic relapsing cholangitis, and the latter has been suspected as a predisposing factor for the late development of cholangiocarcinoma.[58] The cells of the dilated walls might have already proceeded to the precancerous stage at the time of the initial operation, and might have undergone genetic changes during the postoperative periods.[73] Therefore, careful follow-up is strongly recommended.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Extrahepatic bile duct measurement by using transabdominal ultrasound in Japanese adults: multi-center prospective study

Takao Itoi · Terumi Kamisawa · Hideki Fujii · Kazuo Inui · Hiroyuki Maguchi · Yoshinori Hamada · Toshiaki Nakano · Hisami Ando · Tsugumichi Koshinaga · Keiko Shibagaki · Taminori Obayashi · Yasuhiko Miyazawa

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Abstract
Background In adults, less than 10-mm bile duct has idiomatically been recognized as "non-dilated bile duct" though there was no obvious evidence. The aim of this study was to prospectively examine the maximum inner diameter of extrahepatic bile duct (MDEBD) in consecutive adults.
Methods Transabdominal ultrasound (US) was performed to measure the MDEBD of 8840 cases (4420 male) in five institutions. The frequency of ultrasound probe ranged from 3.5 to 5 MHz.
Results The mean diameter of MDEBD was 4.5 ± 1.4 mm (range 1–14 mm). The relationship between the MDEBD and age was shown as follows: MDEBD = 2.83 + 0.03 × age. Multiple regression analysis was analyzed between 6 groups and significant α level is 0.008 in this analysis. In all age groups but 20s and 30s, there was statistically significant MDEBD among each age group ($p < 0.0001$). Mean, mode value and median MDEBD is increasing according to the age as follows: 20s: 3.9 ± 1.0 mm, 30s: 3.9 ± 1.2 mm, 40s: 4.3 ± 1.2 mm, 50s: 4.6 ± 1.3 mm, 60s: 4.9 ± 1.4 mm, >70s: 5.3 ± 1.6 mm.
Conclusion The present study revealed that MDEBD positively correlates with age. Therefore, when we examine the presence of dilation of the bile duct, our calculating formula appears to be suitable for accurate evaluation.

T. Itoi (✉)
Department of Gastroenterology and Hepatology, Tokyo Medical University, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
e-mail: itoi@tokyo-med.ac.jp

T. Kamisawa
Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

H. Fujii
First Department of Surgery, University of Yamanashi, Kofu, Japan

K. Inui
Department of Gastroenterology, Second Teaching Hospital, Fujita Health University, Nagoya, Japan

H. Maguchi
Center for Gastroenterology, Teine-Keijinkai Hospital, Sapporo, Japan

Y. Hamada
Department of Pediatric Surgery, Kansai Medical University Hirakata Hospital, Osaka, Japan

T. Nakano
University Information Center, Kansai Medical University, Osaka, Japan

H. Ando
Department of Pediatric Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan

T. Koshinaga
Department of Pediatric Surgery, Nihon University School of Medicine, Tokyo, Japan

K. Shibagaki
Health Care Center, Tokyo Medical University, Tokyo, Japan

T. Obayashi
Department of Laboratory Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

Y. Miyazawa
Yamanashiken Koseiren Health Care Center, Yamanashi, Japan

Keywords Extrahepatic bile duct · Ultrasound · Pancreaticobiliary maljunction

Abbreviations

MDEBD	The maximum inner diameter of extrahepatic bile duct
US	Ultrasound
PBM	Pancreaticobiliary maljunction
ERCP	Endoscopic retrograde cholangiopancreatography
PTC	Percutaneous transhepatic cholangiography
CT	Computed tomography
MRI	Magnetic resonance imaging
MRCP	Magnetic resonance cholangiopancreatography

Introduction

Bile duct dilatation indicates abnormal biliary pathology and includes congenital deformities such as pancreaticobiliary maljunction (PBM), and secondary dilatation due to the presence of obstructive mechanisms, such as bile duct stones and benign and malignant strictures. PBM is an anomalous condition in which the pancreatic duct and bile duct merge outside the wall of the duodenum [1]. It causes a continuous reciprocal reflux of pancreatic juice and bile, is a high-risk factor for biliary tract cancer [2, 3]. Thus, since PBM has been thought to be a precancerous condition, surgical treatment of resection of the gallbladder or extrahepatic bile duct, or both, is usually performed regardless of presence of malignancy. PBM includes cases where the bile duct is dilated (PBM with biliary dilatation, congenital choledochal cyst) and those in which it is not (PBM without biliary dilatation). In a nationwide survey carried out by the Japanese Study Group on PBM over 10 years from 1990 to 1999, 1627 PBM cases were examined in detail [4]. These results showed that biliary tract cancer was detected in 278 of the 1627 cases (17.1 %). Of these, in the 1239 cases of PBM with biliary dilatation, there were 131 (10.6 %) cases with concomitant biliary tract cancer, which was located in the gallbladder in 85 (64.9 %) cases, the bile duct in 44 (33.6 %) cases, and cancer of unknown origin in 2 cases. In contrast, of the 388 cases of PBM without biliary dilatation, there were 147 (37.9 %) cases with concomitant biliary tract cancer, which was located in the gallbladder in 137 (93.2 %) cases and in the bile duct in 10 (6.8 %) cases. These results show that PBM with biliary dilatation has a potential of malignant transformation, not only in the gallbladder but also in the dilated bile duct and that PBM without biliary dilatation has a potential of malignant transformation mainly in the gallbladder. Thus, several investigators have advocated that radical prophylactic resection of the extrahepatic bile duct in patients with PBM without biliary dilatation should not be recommended [5]. Thus, diagnosis of dilated or non-dilated bile duct is extremely important for the selection of surgical therapy. Some investigators have advocated that the criterion of non-dilation of the bile duct is less than 6 mm [6] or 8 mm [7] in childhood, but there is no obvious evidence for the criterion. On the other hand, in adult, less than 10-mm bile duct is idiomatically call as "non-dilated bile duct", but there is not accumulated data at all.

The aim of this study was to prospectively examine the maximum inner diameter of extrahepatic bile duct (MDEBD) in consecutively seen adults in whom no hepatobiliary-pancreato diseases were recognized on history or using transabdominal ultrasound (US) in several Japanese centers.

Patients and methods

A prospective, multicenter study was carried out from October 2010 to April 2011 in the following five institutions in Japan: Tokyo Medical University, Tokyo Metropolitan Komagome Hospital, University of Yamanashi, Fujita Health University Second Teaching Hospital and Teine-Keijinkai Hospital. The maximum inner diameter (integral value) of the extrahepatic bile duct was measured in adults more than 16 years old using transabdominal US by routine right hypochondriac oblique scan under fasting condition (Fig. 1). The frequency of the ultrasound probe ranged from 3.5 to 5 MHz. Cases with any history of hepatobiliary-pancreato diseases or abnormal findings in the liver, biliary tract and pancreas on US were excluded.

The study was approved by the institutional review board of Tokyo Metropolitan Komagome Hospital and was in compliance with the Declaration of Helsinki.

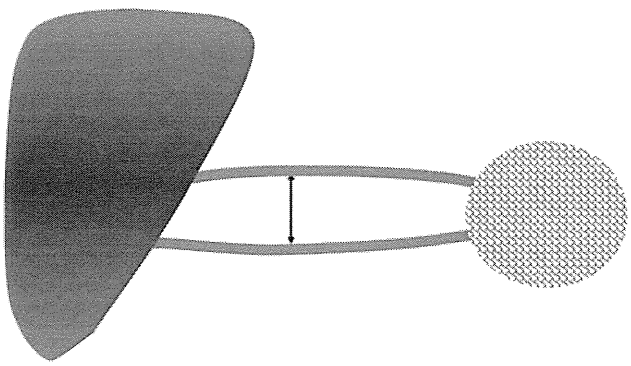

Fig. 1 Measurement of the maximum inner diameter of extrahepatic bile duct. The maximum inner diameter of extrahepatic bile duct was measured by transabdominal ultrasound

Statistical analysis

All analyses were performed with SAS software, JMP® 9.0 (SAS Institute, Cary, NC). The Kruskal–Wallis test was used a non-parametric statistic to compare the diameters of bile duct in each institutions. A p value of less than 0.05 was regarded to indicate a statistically significant difference. Multiple regression analysis was used for multivariate analysis. The Kruskal–Wallis test was also used for multiple comparisons after setting of α level according to the Bonferroni method.

Results

Patient characteristics

A total of 9859 cases were collected from 5 institutions. Since the results of a pilot study revealed that there was statistically significant distributions in age between male ($n = 5024$) and female ($n = 4835$) (52.2 ± 0.18 and 50.3 ± 0.18, respectively, $p < 0.0211$) and MDEBD (4.54 ± 1.36 and 4.47 ± 1.43, respectively, $p < 0.0001$), we analyzed this study after distribution was matched to gender to avoid a bias in the number of cases related to gender. In the final analysis, 8840 cases (4420 males) in five institutions were extracted and analyzed in this study. The mean age was 51.6 years (range 20–90 years) in this group.

MDEBD and distribution

The mean diameter of MDEBD was 4.5 ± 1.4 mm (range 1–14 mm) and the median diameter of MDEBD was 4 mm. As MDEBD did not show normal distribution (Fig. 2), in this study we used a non-parametric test for statistical analysis.

Univariate analysis of MDEBD

Univariate analysis was conducted using age, gender and institution as the explanatory variable and MDEBD values were variable.

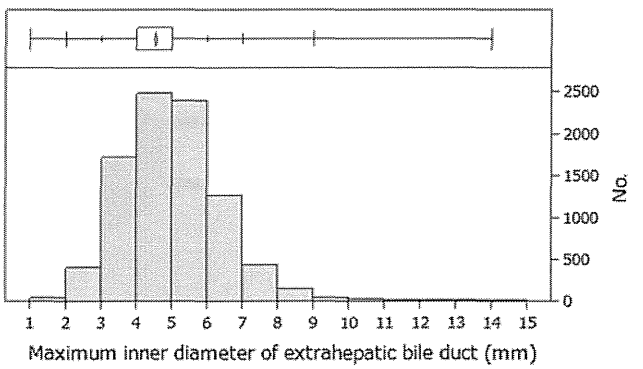

Fig. 2 Distribution of maximum inner diameter of bile duct

Age

There was a statistically positive correlation between age and MDEBD ($r = 0.29$, $p < 0.0001$) (Fig. 3). The relationship between the MDEBD and age was shown as follows: MDEBD = 2.83 + 0.03 × age (red line in Fig. 3).

Gender

There was no statistically significant difference between gender and MDEBD (male 4.5 ± 1.4 mm, female 4.5 ± 1.4 mm, $p = 0.8581$).

Institution

The mean of MDEBD for all institutions was 3.8 ± 1.3 mm (95 % CI 3.71–3.81) (Institution A), 5.3 ± 1.4 mm (95 % CI 5.17–5.37) (B), 4.9 ± 1.2 mm (95 % CI 4.89–4.98) (C), 4.5 ± 1.3 mm (95 % CI 4.45–4.59) (D), and 4.5 ± 1.3 mm (95 % CI 4.45–4.58) (E), respectively (Table 1). There was a statistically significant difference among each group, except for Institutions D versus E ($p < 0.0001$).

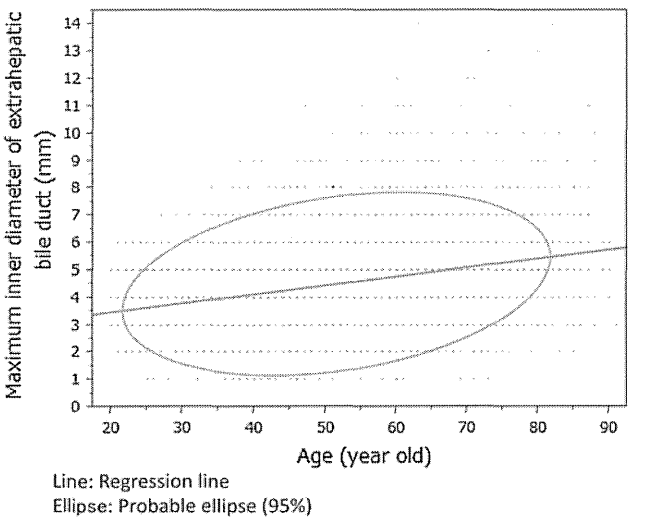

Fig. 3 Bivariate analysis between age and bile duct diameter. *Green* bivariate normal ellipse, *red* applying straight line

Table 1 Bivariate analysis between age and bile duct diameter

Institution	No. of cases	EHBD (mm, mean ± SD)	95 % CI
A	2319	3.8 ± 1.3	3.71–3.81
B	619	5.3 ± 1.4	5.17–5.37
C	2838	4.9 ± 1.2	4.89–4.98
D	1392	4.5 ± 1.3	4.45–4.59
E	1672	4.5 ± 1.3	4.45–4.58

Table 2 Outcome of statistical significant parameters by multivariate analysis of MDEBD

Parameter	EV	SE	t value	*p*	95 % CI
Institution A	−0.631	0.026	−26.93	<.0001	−0.683 to −0.579
Institution B	0.417	0.042	9.37	<.0001	0.333 to 0.501
Institution C	0.441	0.024	18.00	<.0001	0.394 to 0.488
Institution D	−0.162	0.030	18.00	<.0001	−0.221 to −0.104
Age	0.027	0.001	21.22	<.0001	0.024 to 0.029

EV estimate value, *SE* standard error, *CI* confidence interval

Multivariate analysis of MDEBD

Multivariate analysis was conducted using age and institution as the explanatory variable, which showed statistical significance in univariate analysis. Multiple regression analysis suggested that age and institution were independent explanatory variables (Table 2).

Relationship between age and MDEBD in the five institutions

Based on the results of multivariate analysis, simple comparison between age and MDEBD is not suitable because of the presence of the independent factor of institution. Therefore, the relationship between age and MDEBD in each institution was examined (Fig. 4). These results suggested that there is no statistically significant difference between age and distribution of MDEBD, though there are statistically significant differences in the MDEBD in each institution. The relationship between age and MDEBD showed positive and similar correlations in each institution (slope 0.021–0.030). The total correlation coefficient (0.29) was relatively superior to that of each institution (A 0.22, B 0.22, C 0.20, D 0.32, E 0.24). In addition, estimated values in each institution (−0.6 to 0.4) (Table 2) obtained from multivariate analysis were all within 1.0. Based on these results, the discrepancy among each institution did not appear to affect correlation between age and MDEBD.

Relationship between age and MDEBD

The relationship between age and MDEBD are shown in Table 3. If the upper limit of diameter of bile duct were to be hypothesized as follows: MDEBD + 2 × SD, upper limits of diameter of bile duct in each age are shown in Table 3.

One-way analysis of variance showed there was no normal distribution between stratified age group and MDEBD (Fig. 5). Multiple regression analysis was analyzed between 6 groups and significant α level is 0.008 in this analysis. In all age groups but the 20s and 30s, there was statistically significant MDEBD among each age group

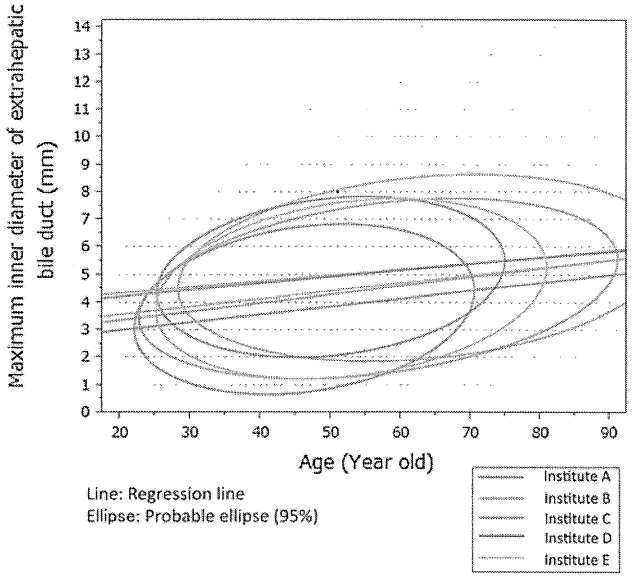

Fig. 4 Relationship between age and MDEBD in 4 institutions. Institutions: *red* Fujita Health University, *green* Tokyo Metropolitan Komagome Hospital, *blue* Tokyo Medical University, *orange* University of Yamanashi

($p < 0.0001$). Mean, mode value and median MDEBD is increasing according to the age. These results suggested that MDEBD positively correlated with age.

Discussion

We set out to determine whether MDEBD was related to age. The present study revealed that MDEBD between the 20s and 70s or more increased with age. To the best of our knowledge, this is the first report on the prospective evaluation of MDEBD in a large case series. When the MDEBD is assessed, the measurement of the natural diameter of the bile duct is mandatory. For example, cholangiography by endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC) may not show the true diameter of the bile duct because of the injection of the contrast medium into the bile duct, leading to dilation of the bile duct. In general, US, standard computed tomography (CT) and magnetic resonance imaging (MRI)/magnetic resonance cholangiopancreatography (MRCP) are considered the ideal modalities to measure the MDEBD. However, CT and MRI/MRCP are more expensive than US. Furthermore, there are several issues concerning CT and MRI, e.g., radiation exposure, iodine allergy, and the presence of intraabdominal metal which limits the use of MRI. Therefore, since CT and MRI are not suitable for evaluation of diameter of the bile duct, we selected US. US has been frequently used for screening and is currently very

Table 3 Age-specific statistical values in MDEBD

Age (years)	No. of case	MDEBD	99.2 % CI	Median	Mode	Upper limit (mm)[a]
20–29	138	3.9 ± 1.0	3.62–4.09	4	4	5.9
30–39	1272	3.9 ± 1.2	3.85–4.02	4	4	6.3
40–49	2818	4.3 ± 1.2	4.22–4.34	4	4	6.7
50–59	2234	4.6 ± 1.3	4.56–4.71	5	5	7.2
60–69	1604	4.9 ± 1.4	4.79–4.97	5	5	7.7
≥70	776	5.3 ± 1.6	5.12–5.42	5	5	8.5

MDEBD maximum inner diameter of extrahepatic bile duct (mm, mean ± SD), *CI* confidence interval

[a] Upper limit of diameter of bile duct (mm): MDEBD + 2 × SD

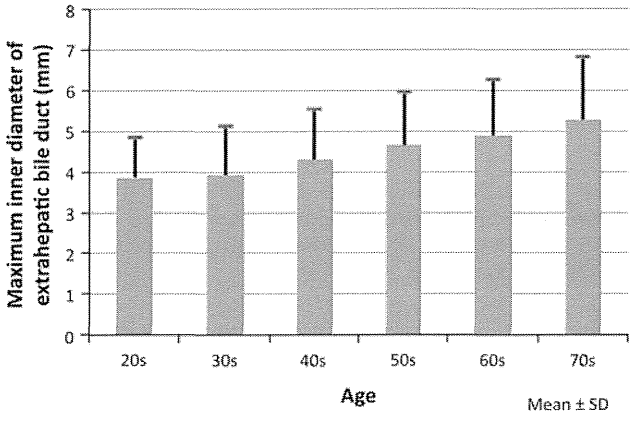

Fig. 5 One-way analysis of variance of age-specific MDEBD

popular in Japan. Thus, we were able to accumulate a relatively large number of cases during short term. In fact, several investigators previously examined the diameter of the bile duct by means of US [8, 9]. Based on MDEBD analysis of 1018 adults (60-96 years), Parret et al. [8] reported that the MDEBD in 60 year old and 85 year old was 3.6 and 4.0 mm, respectively. Admassie et al. [9] reported that the MDEBD in 293 adults (the mean age 35.8 years, range 15–75) was 3.9 mm and that MDEBD increases with age. Although total number of cases in the present study was higher than two previous studies, these results were relatively similar to our results.

Until now, bile ducts less than 10 mm in adults have been considered to be "non-dilated bile ducts". Although according to our results, a 10-mm bile duct should be a dilated bile duct, accurate diagnosis of dilation of the bile duct should be determined based on the MDEBD in each age group. In fact, our results suggest that MDEBD is comparatively lower than 10 mm regardless of age in adults. If the upper limit of the diameter of the bile duct was recognized as a landmark of dilation of the bile duct, criteria of dilation of the bile duct would become more clear as follows: 20s: 5.9 mm, 30s: 6.3 mm, 40s: 6.7 mm, 50s: 7.2 mm, 60s: 7.7 mm, > 70s: 8.5 mm (Table 3).Using these criteria, the number of PBM without biliary dilatation may decrease whereas many cases of PBM may be diagnosed as "biliary dilatation type", resulting in prophylactic resection of not only gallbladder but also extrahepatic bile duct.

For children 15 years of age or less, 5 mm and below has been thought to be dilation of the bile duct in Japan. However, this cut-off criterion is used without obvious evidence as well as in adults. Recently, we have reported the standard value of MDEBD of 740 children according to age (monthly), body weight, and body height [10]. As a result, we clarified that MDEBD in children correlated with age and suggested that it is necessary to measure using the following calculation formula: MDEBD = 1.64 + 0.014 × age (in months) − (3.26e-5) × (age in months − 63.0)^2 [10]. Thus, as in children, we may recommend that MDEBD in adults should be calculated using our calculation formula when accurate MDEBD is needed to decide on the therapeutic strategy.

In the present study, eligible adults were accumulated from five institutions. In cases of multi-center studies, multiple sonographers and differences in capability of depiction of the extrahepatic bile duct can cause possible errors among the institutions. The univariate and multivariate analysis showed that not only age, but also institutions, were independent parameters for MDEBD. We concluded that the margin of differences among each institution would not affect the relationship between age and MDEBD. However, since the coefficient of correlation shows the increase in the analysis of the total correlation coefficient (0.29) compared to those of individual analysis (0.22–0.32), errors among each institutions do not essentially affect the results of relationship between age and MDEBD. These results suggest that evaluation of dilation of the bile duct can be conducted using our calculation formula of (MDEBD = 2.83 + 0.03 × age) in any institutions.

In PBM, whether the extrahepatic bile duct shows dilation or not is very important when considering prophylactic bile duct resection in order to avoid the acquired

bile duct cancer. Our results suggest that it seems to be better to define the non-dilated bile duct strictly, though in adults less than 10-mm bile duct has been considered idiomatically as "non-dilated bile duct". This may be able to lead to decrease of bile duct cancers after only prophylactic cholecystectomy in patients with PBM without biliary dilatation.

This study has several limitations. Firstly, the depiction of the extrahepatic bile duct by means of US can be affected by subject factors, e.g., obesity, abnormal conditions like Chilaiditi syndrome (which is one of the causes of a pseudopneumoperitoneum and occurs when bowel gas is interposed between the liver and the hemidiaphragm), and the sonographer's skill. Secondly, although we checked history of hepatobiliary-pancreato diseases, it may be impossible to exclude current asymptomatic hepatobiliary-pancreato diseases, e.g., debris or small stones in the lower bile duct and ampullary tumor.

The present multicenter prospective study revealed that MDEBD is positively correlated with age. Therefore, when we examine the presence of dilation of the bile duct, our calculation formula appears to be suitable for accurate evaluation.

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Conflict of interest We declare that we have no conflict of interest in this publication.

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ORIGINAL ARTICLE

Immunogenicity of inactivated seasonal influenza vaccine in adult and pediatric liver transplant recipients over two seasons

Michio Suzuki[1], Yuka Torii[1], Jun-ichi Kawada[1], Hiroshi Kimura[2], Hideya Kamei[3], Yasuharu Onishi[3], Kenitiro Kaneko[4], Hisami Ando[4], Tetsuya Kiuchi[3] and Yoshinori Ito[1]

[1]Department of Pediatrics, , [2]Department of Virology, [3]Department of Transplant Surgery, Nagoya University Graduate School of Medicine and [4]Department of Pediatric Surgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

ABSTRACT

Immunological responses to influenza vaccination administered to liver transplantation recipients are not fully elucidated. To compare inactivated influenza vaccine's immunogenicity between adult and pediatric recipients, 16 adult and 15 pediatric living donor liver transplantation recipients in the 2010–11 influenza season, and 53 adult and 21 pediatric recipients in the 2011–12 season, were investigated. Seroprotection rates (hemagglutinin-inhibition [HI] antibody titer 1:40) were 50–94% to all three antigens among adults and 27–80% among children in both seasons. Seroconversion rates (fourfold or more HI antibody rise) were 32–56% among adults and 13–67% among children in both seasons. No significant differences were observed between the two groups. In addition, 20/53 adult and 13/21 pediatric recipients received a vaccine containing identical antigens in both of these seasons. Geometric mean titer fold increases of all three antigens in adult recipients were significantly lower than those in recipients who had not received a preceding vaccination. In contrast, in pediatric recipients, there were no significant differences between the groups who had and had not received preceding vaccinations. The number of patients with rejection did not differ significantly between the two groups (0/53 vs. 1/21) in the 2011–12 season. The incidence of influenza after vaccination was significantly different between adult and pediatric recipients (0/16 vs. 5/15 in 2010–11 and 0/53 vs. 3/21 in 2011–12, respectively). Overall, there were no significant differences in antibody responses between adult and pediatric groups. Influenza infection was more frequent in pediatric recipients. Long-term response to preceding vaccinations appeared to be insufficient in both groups.

Key words immunogenicity, influenza vaccination, liver transplantation.

Influenza is a highly infectious viral illness. Because solid organ transplant recipients are treated with immunosuppressive drugs that affect the immune system (1), these patients are at high risk of influenza-related complications, including pneumonia, sepsis, central nervous system disorders and acute graft rejection (2–5). The World Health Organization, the Advisory Committee on Immunization Practices and the American Society of

Correspondence:
Yoshinori Ito, Department of Pediatrics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.
Tel: +81 52 744 2294; fax: +81 52 744 2974; email: yoshi-i@med.nagoya-u.ac.jp

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List of Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GMT, geometric mean titers; HI, hemagglutination inhibition.

Transplantation/Transplant Surgeons recommend annual administration of the seasonal inactivated influenza vaccine in post-transplant recipients (6). Several previous reports have evaluated influenza vaccination in post-liver transplantation adults (7–10) and children (11–13). We also previously reported safety and immunogenicity of influenza vaccination administered to pediatric living donor liver transplant recipients for four seasons (14, 15). Although these reports support the safety of inactivated influenza vaccine, evidence about its effectiveness is not conclusive. Safety and effectiveness depend on various factors, such as immune status of recipients, recipient's age, type of vaccine and inoculation method. In terms of age group, the summary efficacy of inactivated influenza vaccines in children was shown to be less than that in adults in a systematic review with meta-analyses of controlled trials (16). Clarifying the differences between adult and pediatric recipients may provide useful information for planning of additional strategies to prevent influenza in pediatric recipients. Because there are fewer studies of influenza vaccination in pediatric than in adult transplant recipients, assessment, and comparison of safety and immunogenicity of trivalent inactivated influenza vaccine between adult and pediatric living donor liver transplant patients in a single hospital is valuable. Moreover, the vaccine administered in our country included identical influenza antigens through the 2010–11 and 2011–12 seasons, allowing evaluation of long-term persistence of antibody responses and the contribution of preceding vaccinations to boosting responses.

PATIENTS AND METHODS

Study design

During the 2010–11 and 2011–12 influenza seasons, adult (≥16 years) and pediatric (<16 years) living donor liver transplant recipients were enrolled in this study. All recipients were followed up at the Nagoya University Graduate School of Medicine. Information about each participant's underlying illness was collected by reviewing their medical records. Recipients were advised to receive the influenza vaccination if 12 months had elapsed since transplantation. Exclusion criteria for the study were interval of less than 6 months since acute rejection treatment and allergy to egg proteins. Before vaccination, serum concentrations of immunoglobulin A, M and G were measured in all patients and found to be within the normal range. Each vaccine dose was administered as an s.c. injection according to the guidelines in Japan. Patients aged <13 years were given two doses and those aged >13 years one dose of the vaccine. In the 2010–11 season, the doses were 0.2 mL for patients aged 1–5 years, 0.3 mL for those aged 6–12 years (two doses 2–4 weeks apart) and 0.5 mL for those aged ≥13 years. In the 2011–12 season (the guidelines changed between seasons), the doses were 0.25 mL for patients aged 1–3 years; 0.5 mL for those aged 3–12 years (two doses) and 0.5 mL for patients aged ≥13 years. Blood samples for HI testing were obtained at baseline and 4–6 weeks after vaccination (for those who had received one dose of vaccine) or 6–10 weeks after the first vaccination (for those who had received two doses of vaccine). The study design and purpose was approved by the institutional review board of Nagoya University and fully explained to all patients and/or guardians, and informed consent was obtained prior to enrollment.

Sixteen adult and 15 pediatric living donor liver transplant recipients in the 2010–11 season, and 53 adults and 21 children in the 2011–12 season met the above eligibility criteria. Of the 53 adults investigated in the 2011–12 season, 20 had received the same influenza vaccination in the preceding season (2010–11 and 2011–12). Of the 21 children investigated in the 2011–12 season, 13 had received the same influenza vaccination in the preceding season (2010–11 and 2011–12). Therefore, 20 adult and 13 pediatric patients who had received a vaccination in the former influenza season were evaluated for vaccine efficacy over both seasons.

Vaccines

The trivalent influenza vaccine used was derived from A/California/7/2009pdm (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008. The antigens included in the vaccine were identical over the two influenza seasons. The commercial vaccines (Kitasato Institute Research Center for Biologicals, Saitama, Japan in the 2010–11 season, and Kaketsuken, Kumamoto, Japan in the 2011–12 season) were unadjuvanted, inactivated, split-virus vaccines containing >15 μg of hemagglutinin antigen per 0.5 mL.

Safety assessment

Information regarding the safety of each vaccine was collected at 4 weekly intervals for 6 months post-vaccination. Serum hepatobiliary enzymes were measured as markers of acute allograft rejection. All subjects were assessed for acute febrile illness (>38.0°C) and influenza virus infection was checked for by viral antigen testing (QuickNavi-Flu; Denka-Seiken, Tokyo, Japan) of nasopharyngeal swabs obtained during each acute febrile illness.

Immunogenicity assessment

Influenza-specific HI antibodies for the three influenza strains included in the vaccine were measured at a

commercial laboratory (SRL, Tachikawa, Japan). Immunogenicity was evaluated in three ways: seroprotection rates, defined as the percentage of subjects achieving an HI titer $\geq$40; seroconversion rates, defined as the percentage of subjects achieving at least a fourfold increase in HI titers from seropositive prevaccination titer ($\geq$10) or rise from $<$10 to $\geq$40 in those who were seronegative prevaccination; and HI GMT to each influenza antigen at baseline and post-vaccination.

Statistical analysis

Statistical significance for sex, seroprotection rate, and seroconversion rate was assessed by the X^2 test or Fisher's exact test (for small samples). For results expressed as median (age, follow-up years, trough concentration of FK506 and geometric mean titer), statistical analysis was performed with the Mann–Whitney *U*-test. For univariate analysis, the X^2 test was used to compare categorical variables. Logistic regression was used for multivariate analysis. A *P*-value ≤ 0.05 was considered statistically significant. All statistical analyses were performed using SPSS statistics version 20 (IBM, Armonk, NY, USA).

RESULTS

Patient characteristics

Sixteen adult and 15 pediatric living donor liver transplant recipients were studied in the 2010–11 season, and 53 adults and 21 children in the 2011–12 season. Patient characteristics are summarized in Table 1. Many patients were being maintained on tacrolimus-based immunosuppressive regimens. A few patients were also receiving mycophenolate mofetil, prednisolone, azathioprine and cyclosporine. The subjects had no history of anaphylactic reactions to vaccines or eggs. In terms of comorbidities, one (6%) and nine (17%) adult recipients in each season had diabetes mellitus.

Safety evaluation

Acute allograft rejection occurred in none of the recipients during the 2010–11 season and in one pediatric recipient during the 2011–12 season. The number of patients with rejection did not differ significantly between adult and pediatric recipients in the 2011–12 season ($P = 0.284$). In the 2010–11 season, a 4-year-old girl's

Table 1. Clinical characteristics of subjects receiving influenza vaccine

	2010–11 season			2011–12 season		
Characteristic	Adults ($n = 16$)	Children ($n = 15$)	*P*-value	Adults ($n = 53$)	Children ($n = 21$)	*P*-value
Male, *n* (%)	5 (31%)	8 (53%)	0.213[§]	22 (42%)	10 (48%)	0.632[§]
Age in years at vaccination, median (range)	53.7 (16.3–70.0)	6.4 (2.6–13.8)		56.0 (17.3–71.0)	5.9 (1.7–14.8)	
Years since transplantation, median (range)	**6.2 (3.8–13.3)**	**3.7 (1.1–12.4)**	**0.021**[¶]	**5.8 (1.0–23.2)**	**3.0 (1.1–13.4)**	**0.006**[¶]
Reason for transplantation						
Primary biliary cirrhosis	4	0		11	0	
Biliary atresia	3	13		8	17	
Hepatitis C	3	0		10	0	
Fulminant hepatitis	2	0		4	0	
Hepatitis B	1	0		13	0	
Other[†]	3	2		7	4	
Immunosuppressive therapy, n						
FK 506	5	12		25	18	
FK 506 and MMF	3	1		10	0	
FK 506 and MMF and PSL	3	0		8	1	
FK 506 and PSL and azathioprine	2	0		1	0	
Cyclosporine	1	1		6	0	
Other[‡]	2	1		3	2	
Trough concentrations of FK 506 in ng/mL; median (range)	3.9 (0.6–7.3)	2.4 (0.9–6.8)	0.051[¶]	**4.8 (0.1–9.1)**	**2.5 (0.6–5.2)**	**<0.001**[¶]

FK 506, tacrolimus; MMF, mycophenolate mofetil; PSL, prednisolone.
Bolded values are statistically significant ($P \leq 0.05$).
[†]other includes Alagille syndrome, alcoholic liver cirrhosis, autoimmune hepatitis, congenital hepatic fibrosis, congenital hypoplasia of the portal vein, hepatoblastoma, liver metastasis of pancreatic solid pseudopapillary tumor, ornithine transcarbamylase deficiency, primary sclerosing cholangitis and Wilson disease. [‡]other includes FK 506 and PSL, FK 506 and interferon β-1b, FK 506 and methylprednisolone, FK 506 and MMF and hydrocortisone, or cyclosporine and MMF. [§]X^2 test. [¶]Mann–Whitney *U*-test.

AST and ALT concentrations increased to 297 and 262 IU/mL, respectively, one day after the first dose of vaccination. However, these values decreased spontaneously and she did not undergo a liver biopsy. In the 2011–12 season, a 2-year-old boy had a mild cough and his AST and ALT concentrations were 80 and 108 IU/mL, respectively, at the time of vaccination. Sixteen days after vaccination, his AST and ALT concentrations increased to 140 and 179 IU/mL, respectively, and he was diagnosed with acute allograft rejection by liver biopsy. He received steroid pulse therapy and did not receive the second vaccination.

Immunogenicity

Immunological responses to influenza vaccine are summarized in Table 2. No significant differences in seroconversion rate or GMT fold increase were observed between adult and pediatric groups in each season. However, adult H1N1 pre-seroprotection rates in the 2010–11 season and post-seroprotection rates in the 2011–12 season were significantly higher than those in the pediatric group. The GMT of all three antigens before vaccination and H1N1 GMT after vaccination in the adult group were significantly higher than in the pediatric group in the 2011–12 season. Interestingly, 20/53 adult and 13/21 pediatric recipients were vaccinated in both seasons with the same vaccine. This allowed comparison of immunogenicity in each season for both patient groups (Table 3). The number of patients who received two consecutive vaccinations did not differ significantly between the adult and pediatric groups ($P = 0.059$). In the adult group, GMT fold increases in all three antigens in the recipients who received two consecutive vaccinations were significantly lower than in those who did not receive vaccination in the 2010–11 season. In contrast, in the pediatric recipients, there were no significant differences between the groups who had and had not received preceding vaccination. Of the recipients who received vaccination in both seasons, 11/20 adults and 8/11 children underwent examination of HI antibody titers pre- and post-vaccination through both seasons. Kinetics of their titers are shown in Fig. 1.

In the 2011–12 season, factors associated with seroprotection were assessed in 74 patients (53 adults and 21 children). Univariate analysis showed that being an adult and number of years since transplantation were significantly correlated with seroprotection. However, this finding was not significant according to multivariate analysis (Table 4). Factors associated with seroprotection

Table 2. Immunologic responses to influenza vaccine over two influenza seasons

	2010–11 season			2011–12 season		
	Adults ($n = 16$)	Children ($n = 15$)	*P*-values	Adults ($n = 53$)	Children ($n = 21$)	*P*-values
H1N1						
Seroprotection rate (pre)	**12 (75%)**	**4 (27%)**	**0.007**[†]	29 (55%)	6 (29%)	0.069[†]
Seroprotection rate (post)	15 (94%)	12 (80%)	0.275[‡]	**43 (81%)**	**12 (57%)**	**0.033**[†]
Seroconversion rate	8 (50%)	10 (67%)	0.347[†]	22 (42%)	7 (33%)	0.516[†]
GMT pre	47.6	18.2	0.138[§]	**37.0**	**13.9**	**0.015**[§]
GMT post	217.0	116.0	0.436[§]	**142.2**	**38.7**	**0.004**[§]
GMT fold increase	4.6	6.4	0.654[§]	3.8	2.8	0.530[§]
H3N2						
Seroprotection rate (pre)	3 (19%)	5 (33%)	0.303[‡]	19 (36%)	4 (19%)	0.159[†]
Seroprotection rate (post)	11 (69%)	10 (67%)	0.602[‡]	39 (74%)	12 (57%)	0.168[†]
Seroconversion rate	9 (56%)	6 (40%)	0.366[†]	23 (43%)	9 (43%)	0.966[†]
GMT pre	12.4	15.9	0.560[§]	**22.8**	**10.3**	**0.014**[§]
GMT post	95.1	66.5	0.380[§]	70.2	38.7	0.135[§]
GMT fold increase	7.7	4.2	0.338[§]	3.1	3.7	0.506[§]
B						
Seroprotection rate (pre)	2 (13%)	6 (40%)	0.090[‡]	**20 (38%)**	**3 (14%)**	**0.049**[†]
Seroprotection rate (post)	8 (50%)	4 (27%)	0.183[†]	39 (74%)	15 (71%)	0.851[†]
Seroconversion rate	6 (38%)	2 (13%)	0.130[¶]	17 (32%)	9 (43%)	0.381[†]
GMT pre	10.4	15.9	0.466[§]	**21.1**	**10.3**	**0.007**[§]
GMT post	29.5	17.4	0.252[§]	51.3	38.7	0.275[§]
GMT fold increase	2.8	1.1	0.054[§]	2.4	3.7	0.069[§]

Bolded values are statistically significant ($P \leq 0.05$).
[†]X^2 test. [‡]Fisher's exact test. [§]Mann–Whitney *U*-test.

Table 3. Immunologic responses to the influenza vaccine in patients receiving identical vaccinations in two consecutive seasons

	Adults			Children			Adults versus children 2010/11–11/12[†]
	2010/11–11/12[†] ($n=20$)	2011/12[‡] ($n=33$)	*P*-value	2010/11–11/12[†] ($n=13$)	2011/12[‡] ($n=8$)	*P*-value	*P*-value
Age in years at vaccination, median (range)	58.0 (17.3–71.0)	54.7 (18.8–69.3)	0.869[§]	**7.1 (3.6–14.8)**	**2.7 (1.7–10.3)**	**0.006**[§]	
Trough concentration of FK 506 in ng/mL; median (range)	**4.6 (1.8–9.1)**	4.8 (0.1–8.6)	0.796[§]	**2.1 (0.9–3.7)**	**3.9 (0.6–5.2)**	**0.045**[§]	**<0.001**[§]
H1N1							
Seroprotection rate (pre)	13 (65%)	16 (49%)	0.242[¶]	5 (39%)	1 (13%)	0.221[‖]	0.135[¶]
Seroprotection rate (post)	15 (75%)	28 (85%)	0.295[‖]	9 (69%)	3 (38%)	0.166[‖]	0.509[‖]
Seroconversion rate	5 (25%)	17 (52%)	0.058[¶]	4 (31%)	3 (38%)	0.557[‖]	0.509[‖]
GMT pre	54.6	29.2	0.137[§]	21.1	7.1	0.089[§]	0.087[§]
GMT post	98.5	177.7	0.117[§]	49.5	25.9	0.374[§]	0.281[§]
GMT fold increase	**1.8**	**6.1**	**0.008**[§]	2.3	3.7	0.500[§]	0.478[§]
H3N2							
Seroprotection rate (pre)	8 (40%)	11 (33%)	0.624[¶]	4 (31%)	0 (0%)	0.119[‖]	0.436[‖]
Seroprotection rate (post)	13 (65%)	26 (79%)	0.270[¶]	**10 (77%)**	**2 (25%)**	**0.029**[‖]	0.371[‖]
Seroconversion rate	6 (30%)	17 (52%)	0.126[¶]	7 (54%)	2 (25%)	0.201[‖]	0.171[¶]
GMT pre	28.3	20.0	0.425[§]	**16.2**	**5.0**	**0.045**[§]	0.265[§]
GMT post	47.6	88.9	0.235[§]	**58.1**	**20.0**	**0.030**[§]	0.813[§]
GMT fold increase	**1.7**	**4.4**	**0.027**[§]	3.6	4.0	0.697[§]	0.068[§]
B							
Seroprotection rate (pre)	9 (45%)	11 (33%)	0.396[¶]	2 (15%)	1 (13%)	0.684[‖]	0.081[‖]
Seroprotection rate (post)	13 (65%)	26 (79%)	0.270[¶]	10 (77%)	5 (63%)	0.410[‖]	0.371[‖]
Seroconversion rate	**3 (15%)**	**14 (42%)**	**0.038**[¶]	5 (39%)	4 (50%)	0.472[‖]	0.132[‖]
GMT pre	18.7	22.7	0.751[§]	11.1	9.2	0.414[§]	0.118[§]
GMT post	**31.4**	**69.1**	**0.031**[§]	40.0	36.7	0.750[§]	0.730[§]
GMT fold increase	**1.7**	**3.0**	**0.039**[§]	**3.6**	4.0	0.804[§]	**0.033**[§]

GMT, geometric mean titer.

Bolded values are statistically significant ($P \leq 0.05$).

[†]Recipients who received consecutive influenza vaccine over the two seasons. [‡]Recipients who received influenza vaccine exclusively in the 2011–12 season. [§]Mann–Whitney *U*-test. [¶]X^2 test. [‖]Fisher's exact test.

or seroconversion in the 2010–11 season, and those associated with seroconversion rate in the 2011–12 season were also assessed. No significant factors were found by multivariate analysis (data not shown).

Surveillance for influenza virus infection

During the post-vaccination period, five pediatric recipients were diagnosed with influenza in the 2010–11 season; viral antigen testing showed that one was infected with type A and four with type B. Three pediatric recipients were infected with influenza in the 2011–12 season; two with type A and one with type B. In contrast, no adult recipient was diagnosed with influenza during either season. There were significant differences between the two groups in the number of influenza patients after vaccination in both seasons ($P=0.018$ in the 2010–11 season and $P=0.021$ in the 2011–12 season). All influenza patients were treated with oseltamivir or zanamivir and recovered without serious complications.

DISCUSSION

Previous reports have demonstrated the effectiveness of inactivated influenza vaccine for liver transplant recipients. However, evidence concerning immunological responses to influenza vaccination in liver transplantation recipients is still not conclusive. Several reports have demonstrated benefits from influenza vaccination; seroprotection rates after vaccination in adult liver transplant patients were 91–92%/77–92%/68–95% for H1N1/H3N2/B respectively (7, 8), and in pediatric recipients 38–75%/38–71%/23–75% for H1N1/H3N2/B, respectively (12–15). GMTs after vaccination in adults were 52–192/56–190/22–55 for H1N1/H3N2/B, respectively (8–10), and titers in children were 57–119/26–98/13–69 for H1N1/H3N2/B, respectively (13–15). GMT fold increases after vaccination in adults were 1.9–10.6/2.4–8.1/2.4–9.3 for H1N1/H3N2/B, respectively (7, 9, 10), and in children 1.3–2.1/1.5–2.6/1.0–3.0, respectively (13, 15). In the present study, seroprotection rates were

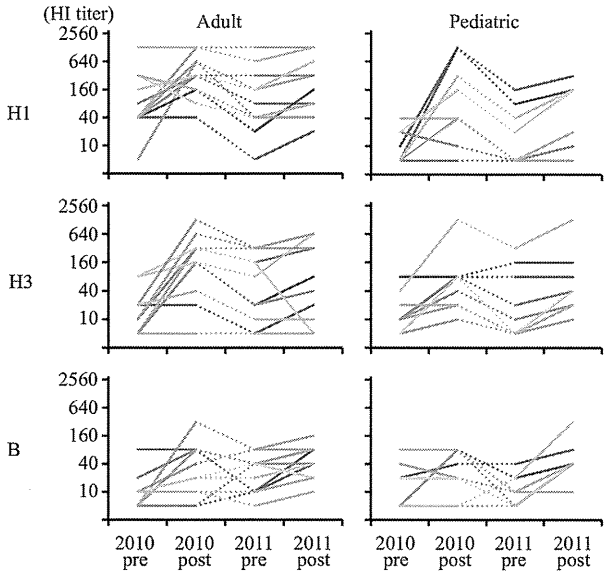

Fig. 1. HI antibody titers against H1/H3/B pre- and post-influenza vaccination in adult and pediatric living donor liver transplant recipients in the 2010–11 and 2011–12 influenza seasons. Serum samples for HI tests were obtained from 11 adults and 8 children.

50–94% to all three antigens among adults and 27–80% among children in both seasons. GMT fold increases after vaccination were 2.8–7.7 for all three antigens in adults and 2.4–3.8 in children. These results are similar to the above-mentioned previous results, suggesting that inactivated influenza vaccine was effective in the two seasons studied in our hospital. In addition, although the cited previous studies did not compare data between adult and pediatric liver transplant recipients in a single study, antibody responses in adult recipients appear to be higher than in pediatric recipients. Mack *et al.* reported that younger age and shorter post-transplant time are risk factors for poor vaccine response (11). The present study was carried out in a single hospital with the same vaccine and may therefore provide more definitive information regarding differences between adult and pediatric liver transplant recipients. Our data show that immunogenicity of influenza vaccination to adult living donor liver transplant recipients was generally not significantly higher in comparison with pediatric recipients in either the 2010–11 or the 2011–12 season.

However, our study has some limitations. First, because healthy controls were not included, we could not compare recipients with healthy controls. Second, the study included relatively few patients.

The virus strains in the 2011–12 seasonal influenza vaccine were identical to those in the 2010–11 vaccine. Therefore, in this study, we were able to investigate long-term persistence of antibodies induced by influenza vaccine, and antibody responses in patients who had and had not been vaccinated in the preceding year. Annual repeated vaccination may have a cumulative effect and be a confounding factor in the magnitude and duration of antibody reactions; however, such effects are difficult to analyze. Several reports have demonstrated that

Table 4. Factors associated with seroprotection in the 2011–12 season

Factor	No. of patients (with factor/total)	No. of patients (with seroprotection/ with factor; %)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
H1N1						
Group (adult)	53/74	43/53 (81.1)	**3.23 (1.07–9.73)**	**0.033**	**3.23 (1.07–9.73)**	**0.038**
Vaccine schedule (1 dose)	55/74	44/55 (80.0)	2.91 (0.94–8.96)	0.057		
Follow up years after transplantation (>5.4 years†)	38/74	32/38 (84.2)	**3.01 (1.00–9.11)**	**0.045**		
FK 506 trough (>4.2 ng/mL†)	33/67	27/33 (81.8)	2.15 (0.69–6.73)	0.186		
H3N2						
Group (adult)	53/74	39/53 (73.6)	2.09 (0.73–6.02)	0.168		
Vaccine (1 dose)	55/74	40/55 (72.7)	1.94 (0.65–5.75)	0.228		
Follow up years after transplantation (>5.4 years†)	38/74	28/38 (73.7)	1.58 (0.59–4.27)	0.363		
FK 506 trough (>4.2 ng/mL†)	33/67	24/33 (72.7)	1.46 (0.51–4.12)	0.479		
B						
Group (adult)	53/74	39/53 (73.6)	1.11 (0.36–3.44)	0.851		
Vaccine (1 dose)	55/74	41/55 (74.5)	1.35 (0.43–4.23)	0.604		
Follow up years after transplantation (>5.4 years†)	38/74	28/38 (73.7)	1.08 (0.39–3.01)	0.887		
FK 506 trough (>4.2 ng/mL†)	33/67	24/33 (72.7)	0.82 (0.27–2.47)	0.725		

CI, confidence interval; FK 506, tacrolimus; OR, odds ratio.
Bolded values are statistically significant ($P \leq 0.05$).
†Median.