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ORIGINAL ARTICLE

Nationwide Survey of the Outcomes of Living Donor Liver Transplantation for Hepatoblastoma in Japan

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Recently, liver transplantation (LT) has been increasingly performed for unresectable hepatoblastoma (HB) with acceptable results. We conducted a national survey of cases undergoing living donor liver transplantation (LDLT) for HB to evaluate their outcomes. Thirty-nine patients (28 males and 11 females with a median age at LDLT of 3.6 years) who had undergone LDLT for HB by the end of 2009 were enrolled in this study. The clinical data were collected from their medical records via a questionnaire survey in 2011 (median follow-up = 4.6 years). Thirteen patients (33.3%) had extrahepatic lesions before LDLT. Thirty-eight patients (97.4%) received chemotherapy, and 15 (38.5%) underwent hepatectomy before LDLT. Twenty-seven patients (69.2%) were alive without recurrence after LDLT, and 12 patients (30.8%) suffered from recurrence. The most common site of recurrence was the lung (9 cases), which was followed by the graft liver (6 cases). The median interval from LDLT to recurrence was 5.5 months. Four of the 9 cases (44.4%) with lung metastasis underwent surgical resection, and 3 were alive without any tumor recurrence. Eight patients died because of tumor recurrence. The multivariate landmark analysis revealed that the independent recurrence risk factors were a high alpha-fetoprotein (AFP) level at diagnosis [≥500,000 ng/mL; hazard ratio (HR) = 7.86, $P = 0.010$], the presence of extrahepatic lesions before LDLT (HR = 3.82, $P = 0.042$), and a high AFP level at LDLT (≥4000 ng/mL; HR = 9.19, $P = 0.036$). The actuarial 3- and 5-year patient survival rates were 84.3% and 77.3%, respectively. In conclusion, with appropriate timing for scheduled LT, LDLT provides a valuable alternative treatment with excellent results for children with HB. *Liver Transpl 20:333-346, 2014.* © 2013 AASLD.

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Abbreviations: ACR, acute cellular rejection; AFP, alpha-fetoprotein; auto-SCT, autologous hematopoietic stem cell transplantation; CBDCA, carboplatin; CI, confidence interval; C-index, concordance index; CITA, cisplatin and tetrahydropyranyl-doxorubicin; CNI, calcineurin inhibitor; CPA, cyclophosphamide; CPT-11, irinotecan; HB, hepatoblastoma; Hi-MEC, ifosfamide, etoposide, carboplatin, and melphalan; HiMT, ifosfamide, etoposide, melphalan, and thiotepa; HR, hazard ratio; HV, hepatic vein; ITEC, ifosfamide, carboplatin, tetrahydropyranyl-doxorubicin, and etoposide; IVC, inferior vena cava; JPLT, Japanese Study Group for Pediatric Liver Tumor; LDLT, living donor liver transplantation; LL, left lobe; LLS, left lateral segment; LT, liver transplantation; N/A, not assessed; PD, peritoneal dissemination; POST-TEXT, Posttreatment Extent of Disease; PRETEXT, Pretreatment Extent of Disease; PV, portal vein; PVTT, portal vein tumor thrombus; ROC, receiver operating characteristic; SIOPEL, Childhood Liver Tumour Strategy Group of the International Society of Paediatric Oncology; THP-ADR, tetrahydropyranyl-doxorubicin; VP-16, etoposide.

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Hepatoblastoma (HB) is the most frequent liver tumor of childhood, and it accounted for approximately 1% of pediatric malignant tumors in a previous report.[1] According to a recent report, the incidence of this tumor has increased over time, and HB is almost exclusively seen in children < 5 years old.[2] Despite treatment with chemotherapy and surgical resection, the prognosis of cases with advanced disease, which is defined as a huge tumor or multifocal tumors occupying the entire liver (precluding complete resection) and/or distant metastatic disease, is poor.[3] Although preoperative chemotherapy makes more than 60% of initially unresectable tumors resectable, 20% of these tumors remain unresectable.[4] Liver transplantation (LT) has been indicated for those cases as the only therapeutic option, and previous studies have shown an improvement in the survival rate of 50% to 90% 5 years after LT.[5,6]

Living donor liver transplantation (LDLT) has been established as the primary therapeutic modality for end-stage liver disease in children, especially in Asian countries.[7] A previous study from a Kyoto group showed that LDLT might allow for the optimal timing of LT in cases with unresectable HB because there is no delay between the completion of chemotherapy and the scheduled LT.[8]

We herein report the results of a nationwide survey of outcomes of LDLT for HB in Japan.

PATIENTS AND METHODS

The primary data related to the cases undergoing LDLT for HB in Japan were collected from the registry kept by the Japanese Liver Transplantation Society. Based on the results of the primary data, a more detailed survey was mailed to the 14 institutions that performed LDLT for HB patients. Forty-one patients underwent LDLT between February 1996 and December 2009. Thirty-nine patients were finally enrolled in the current study because 1 patient died of graft failure on postoperative day 5 and another patient underwent LDLT for liver failure after multiple sessions of transarterial chemoembolization and right lobectomy. There was no tumor recurrence at the time of LDLT, and the patient did not exhibit tumor recurrence during the follow-up period after LDLT. The cases were followed until June 2011 with a median follow-up period of 4.6 years (range = 6 months to 15.2 years). The relevant clinical courses, biochemical and hematological data, pathological findings, and radiological images were collected to construct a database, and then they were analyzed by statistical analysis with permission from the institutional review board of the National Center for Child Health and Development and the Japanese Liver Transplantation Society.

Pretransplant Management for HB

The Pretreatment Extent of Disease (PRETEXT) staging system was used for the pretreatment staging of tumors, and the Posttreatment Extent of Disease (POST-TEXT) staging system was used for the post-chemotherapy staging of tumors before any surgical resection on the basis of radiological findings. The pretransplant treatment, such as chemotherapy and surgical resection, was selected by each institution, although the Japanese Study Group for Pediatric Liver Tumor (JPLT) has proposed a nationwide protocol for liver tumors in childhood, and this served as a background guideline.

The current protocol, JPLT-2, has been described elsewhere[9]; in brief, PRETEXT I tumors were primarily resected, and PRETEXT II-IV cases were treated with preoperative chemotherapy. At least 2 courses of a combination of cisplatin and tetrahydropyranyl-doxorubicin (THP-ADR), which was designated CITA, were repeated preoperatively. When CITA failed to induce a partial response, a combination of ifosfamide, carboplatin (CBDCA), THP-ADR, and etoposide (VP-16), which was designated ITEC, was given until the tumor became resectable. Postoperative chemotherapy was used in all cases. PRETEXT III, PRETEXT IV, and metastatic cases were treated with 2 courses of CITA. Patients who required salvage with ITEC preoperatively were treated with 2 courses of ITEC. If a complete response was not obtained at this point, 2 additional courses were added. Metastatic cases were treated with high-dose chemotherapy, which consisted of a combination of ifosfamide, VP-16, CBDCA, and melphalan (designated the Hi-MEC protocol) or a combination of ifosfamide, VP-16, melphalan, and thiotepa (designated the Hi-MT protocol), as proposed by the JPLT group,[10,11] and with autologous hematopoietic stem cell transplantation (auto-SCT), although the metastatic lesions that were considered to be resectable were surgically resected at the discretion of each institution.

LDLT

The indications for and timing of LDLT were left to the discretion of each institution. In each case, after the family's consent to proceed with the operation was obtained, a thorough medical evaluation was performed to determine the suitability of the donor. Donors were selected on the basis of the results of a medical evaluation, including liver function tests, ABO blood group typing, and graft/recipient size matching. The graft type was selected according to the graft/recipient weight ratio or the graft volume/standard liver volume ratio.[7,8,12,13] All of the donors were the children's parents, except for 1 grandfather. The ages of the donors ranged from 23 to 64 years with a median age of 34 years. The blood type combination was incompatible in 2 cases. The graft type was a left lobe (LL) in 6 cases, a left lateral segment (LLS) in 31 cases, and a reduced LLS in 2 cases. The native inferior vena cava (IVC) was completely removed in 4 cases during whole hepatectomy. Thereafter, 1 patient underwent the reconstruction of a new IVC with the vessel graft from his donor's internal

jugular vein (case 29). The other 3 patients did not undergo IVC reconstruction (cases 13, 16, and 18) because they all demonstrated sufficient venous return via collaterals to the azygous systems, which was possibly established by longstanding tumor compression on the IVC; this situation was radiologically confirmed before LDLT, and all patients were verified to be hemodynamically stable by test clamping of the IVC during LDLT. The native IVC wall was partially removed in 2 cases (eg, the anterior wall; cases 34 and 36). Having previously undergone liver resection, 1 patient (case 8) showed an absence of the IVC. The manner of biliary reconstruction was hepaticojejunostomy in 23 cases and duct-to-duct anastomosis in 16 cases. The initial immunosuppression protocol was tacrolimus in all cases except for 1 case in which cyclosporine was administered. Although a target trough level of the calcineurin inhibitor (CNI) was selected at the discretion of each institution on the basis of each patient's condition (eg, renal function), target trough levels of 8 to 10 mg/L for the first 2 weeks, 6 to 8 mg/L for days 15 to 28 after LDLT, and 4 to 6 mg/L from day 29 onward and during posttransplant chemotherapy were maintained in the majority of the cases using tacrolimus. Low-dose steroid therapy, which was basically tapered off by 3 months after LDLT, was used in 25 cases as maintenance immunosuppression, although the corticosteroid was given only intraoperatively at the time of graft reperfusion in 14 cases. The immunosuppression regimen was performed for the 2 cases with a blood type–incompatible combination (cases 18 and 37) in the same manner as that for the cases with a blood type–compatible combination because of the younger age at the time of LDLT.[14] The pathological diagnoses, such as acute cellular rejection (ACR) and chronic rejection, were made according to the Banff criteria.[15] When ACR was confirmed, patients were treated with a high-dose corticosteroid.

Posttransplant Management for HB

The postoperative chemotherapy regimen was selected at the discretion of each institution. If the patient had shown a sufficient response and no dose-dependent side effects of preoperative chemotherapy, the same chemotherapy regimen was adopted postoperatively. If not, then irinotecan (CPT-11) was advocated for use as postoperative chemotherapy.

Statistical Analysis

The tumor recurrence–free survival curves were calculated with the Kaplan-Meier method. The log-rank test was used to evaluate the effects of different characteristics on tumor recurrence. A receiver operating characteristic (ROC) analysis was used to evaluate the ability of the serum alpha-fetoprotein (AFP) level to predict tumor recurrence after LDLT and to choose the optimal cutoff value for the subsequent analysis. To select the optimal cutoff values, the concordance index (C-index) was calculated for each cutoff point on the ROC curve.[16,17] The C-index for a cutoff point was defined as the area of the quadrilateral with vertices on the cutoff point on the ROC curve and points (0, 0), (1, 0), and (1, 1) on the ROC graph. The value estimated the probability that the predictors and the outcomes were concordant. The C-index was calculated with the following formula:

$$\text{C-index} = (\text{Sensitivity} + \text{Specificity})/2$$

We defined the optimal cutoff value as the point showing the highest C-index among the values with a specificity > 0.70. The selected cutoff values were 500,000 ng/mL for AFP at diagnosis (with C-index, sensitivity, and specificity values of 0.78, 0.83, and 0.74, respectively) and 4000 ng/mL for AFP at LDLT (with values of 0.82, 0.92, and 0.74, respectively; Fig. 1). A multivariate Cox regression analysis with backward elimination was used to evaluate the association between tumor recurrence and pretransplant patient characteristics and to estimate the hazard ratio (HR) and its 95% confidence interval (CI). A P value of 0.05 was used for variable selection and was regarded as significant. The IBM SPSS statistics software program (version 19.0, IBM SPSS, Inc., Chicago, IL) was used for the statistical analysis.

RESULTS

The cases included 28 males and 11 females with a median age at the time of diagnosis of 2.5 years, and the ages ranged from 0.2 to 16.6 years. The details of the patient characteristics are summarized in Table 1 (before LDLT) and Table 2 (after LDLT).

Patient Characteristics Before LDLT

The PRETEXT staging was IV in 22 cases (56.4%) and III in 15 cases (38.5%). There was 1 case with PRETEXT stage I (2.6%), and there was 1 case with PRETEXT stage II (2.6%). The median serum AFP level at diagnosis was 375,480 ng/mL, and it ranged from 1835 to 4,400,000 ng/mL. All of the cases, except for 1 case with biliary atresia (case 5) for whom HB was incidentally found during the pathological examination of the explanted native liver, received pretransplant chemotherapy. Each child received 2 to 17 cycles of chemotherapy (median = 6 cycles) before LDLT. The majority of the cases followed the chemotherapy protocol proposed by the JPLT group. A pretransplant chemotherapy protocol containing CITA was the initial protocol for 36 of the 38 cases (94.7%). Twenty of the 28 patients who showed a poor response to chemotherapy with CITA followed an additional protocol using ITEC. Ten patients (26.3%) underwent transarterial chemotherapy with or without embolization before LDLT. Nine patients (23.7%) underwent auto-SCT with high-dose chemotherapy; the Hi-MEC protocol was used in 5 cases, and the Hi-MT protocol was used in 5 cases [this included a case undergoing auto-SCT twice with both high-dose chemotherapy protocols (case 24)]. The indication for

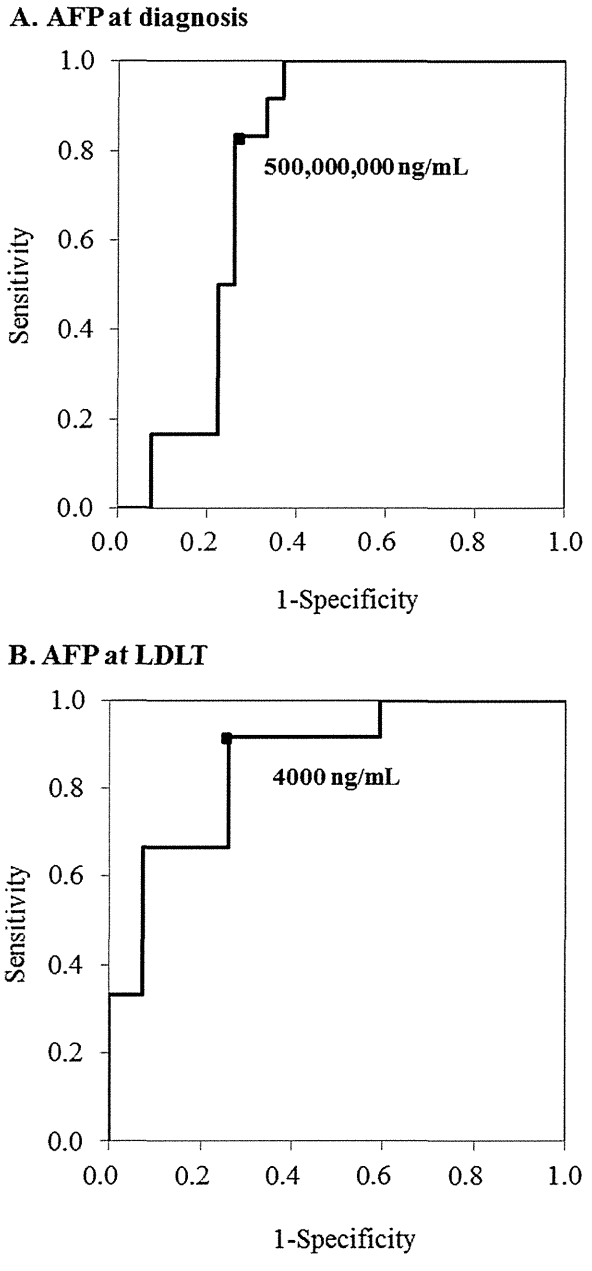

Figure 1. ROC curves for predicting tumor recurrence after LDLT: (A) AFP at diagnosis and (B) AFP at LDLT.

auto-SCT was an unresectable tumor refractory to multiple sessions of conventional-dose chemotherapy after liver resection in 6 cases (cases 6, 9, 13, 14, 24, and 34) and without liver resection in 3 cases (cases 17, 18, and 29). The POST-TEXT staging was IV for 22 cases (62.9%), III for 11 patients (31.4%), and I for 2 patients (5.7%).

Before LDLT, liver resection was performed in 15 cases (38.5%), which included 12 cases after chemotherapy and 3 cases before chemotherapy. Seven of these 15 patients (46.7%) underwent liver resection 2 or 3 times. The types of liver resections included right trisectionectomy (n = 6), right lobectomy (n = 2), left trisectionectomy (n = 3), left hepatectomy (n = 2), right anterior sectionectomy (n = 1), right posterior sectionectomy (n = 1), left lateral segmentectomy (n = 1), and nonanatomical tumor resection (n = 8).

Thirteen patients (33.3%) had extrahepatic lesions before LDLT. One showed direct tumor invasion into the IVC, which was completely resected along with total hepatectomy, at the time of LDLT. Three cases showed tumor invasion into the portal veins (PVs). The tumor thrombus radiologically disappeared after systemic chemotherapy in 1 of the 3 cases (case 33), although the tumor thrombi remained in the other cases at the time of LDLT and were completely removed together with the native portal venous trunk. Two patients showed tumor invasion into other adjacent organs, the stomach and transverse colon, which were completely resected by bowel resection with safe tumor margins. Two patients had a metastatic lesion in the abdominal cavity, which was completely resected 1 and 2 years before LDLT. Two patients had an episode of tumor rupture at the time of onset, and 1 of these patients required urgent hemostasis by transarterial embolization (case 34), although both were confirmed to be free from malignant cells by peritoneal wash cytology at the time of LDLT. Three patients showed lung metastases. One of them underwent partial resection for 4 lesions of lung metastases 3 months before LDLT (case 29), and the metastatic lesions radiologically disappeared after systemic chemotherapy in the other 2 cases.

Patient Characteristics at LDLT

LDLT was primarily indicated because of the presence of an unresectable tumor after systemic chemotherapy (23 cases or 59.0%). The indication for LDLT was unresectable tumor recurrence after hepatectomy, which was once performed for a resectable tumor after systemic chemotherapy, in 15 cases (38.5%). The median age at the time of LDLT was 3.6 years (range = 0.8 to 22.1 years). The mean interval between the diagnosis and LDLT was 1.5 ± 1.8 years (range = 0 days to 6.8 years). The median serum AFP level at LDLT was 3155 ng/mL (range = 10-1,175,690 ng/mL). Seventeen cases (43.6%) showed a less than 2-log decline in the serum AFP level during the period from diagnosis to LDLT, and 2 cases did not show any decline; 1 of the latter 2 cases had an increase in the serum AFP level at LDLT.

Overall Outcomes After LDLT

Thirteen patients (33.3%) experienced more than 1 episode of ACR and were successfully treated with steroid pulse therapy. Eighteen patients (46.2%) developed surgical complications, which included 10 biliary complications (biliary strictures in 8 cases and biliary leakage in 2 cases), 4 cases of intra-abdominal bleeding, and 1 case each of bowel perforation, bowel

TABLE 1. Patient Characteristics Before LDLT

Case Number	Age at Diagnosis (Years)	Sex	PRETEXT Stage	AFP at Diagnosis (ng/mL)	Extrahepatic Lesion(s) (Site)	Chemotherapy Protocol	Cycles of Chemotherapy (n)	POST-TEXT Stage	Liver Resections (n)	Auto-SCT
1	9.7	Male	IV	5942	No	Others*	6	IV	Yes (1)	No
2	0.2	Male	III	677,400	No	CITA/others	6	III	No	No
3	3.7	Male	II	529,000	No	CITA/others	8	N/A†	Yes (1)	No
4	1.8	Male	IV	590,000	Yes (stomach)	CITA*	10	IV	No	No
5	4.6	Female	III	12,900	No	None	N/A	N/A	No	No
6	9.0	Male	IV	2600	No	CITA/others*	7	IV	Yes (1)	Yes
7	2.6	Female	IV	1,500,000	No	CITA*	2	IV	No	No
8	0.4	Male	III	15,000	No	CITA/others	17	N/A†	Yes (1)	No
9	3.6	Male	IV	2,700,000	No	CITA/ITEC	5	III	Yes (3)	Yes
10	3.9	Male	IV	266,000	No	CITA	2	IV	No	No
11	3.2	Male	IV	375,480	Yes (colon)	CITA/ITEC*	16	III	Yes (1)	No
12	11.4	Male	III	36,200	No	CITA	5	III	Yes (1)	No
13	2.5	Female	IV	887,800	Yes (IVC)	CITA/others*	5	IV	Yes (2)	Yes
14	4.0	Male	III	3800	No	Others	8	III	Yes (2)	Yes
15	0.6	Female	III	1,000,000	No	CITA/ITEC	4	IV	No	No
16	0.8	Male	III	4,400,000	No	CITA/ITEC	6	III	No	No
17	4.3	Male	III	1,880,000	No	CITA/ITEC/ others	8	IV	No	Yes
18	1.5	Male	IV	186,699	No	CITA/others	7	III	No	Yes
19	1.1	Female	III	249,400	No	CITA/ITEC	9	IV	No	No
20	11.4	Female	IV	455,700	No	CITA/ITEC/ others	8	III	Yes (1)	No
21	0.5	Female	III	243,800	No	CITA	3	IV	No	No
22	7.9	Male	IV	3000	Yes (omentum)	CITA/others*	15	III	Yes (2)	No
23	3.2	Male	IV	255,840	Yes (PVTT)	CITA/ITEC	4	IV	No	No
24	1.5	Male	III	1,202,849	No	CITA/ITEC/ others	13	III	Yes (2)	Yes‡
25	0.8	Female	IV	2,237,000	No	CITA/ITEC	4	IV	No	No
26	2.2	Male	III	1835	No	CITA	2	III	No	No
27	1.1	Male	III	836,600	No	CITA/ITEC	4	IV	No	No
28	6.9	Male	IV	866,000	Yes (rupture)	CITA/ITEC*	2	IV	No	No
29	1.8	Male	IV	699,700	Yes (lung)	CITA/ITEC	5	IV	No	Yes
30	3.6	Female	IV	723,172	Yes (PVTT)	CITA/ITEC	4	IV	No	No
31	1.1	Female	IV	1,651,000	Yes (lung)	CITA/ITEC	8	IV	No	No
32	1.9	Male	IV	590,000	Yes (lung)	CITA/ITEC	6	IV	No	No
33	2.7	Male	IV	470,500	Yes (PVTT)	CITA	3	IV	No	No
34	16.6	Male	III	5187.6	Yes (rupture)	CITA/ITEC/ others	11	N/A†	Yes (3)	Yes
35	3.8	Male	I	83,470	Yes (lymph node)	CITA/others*	10	I	Yes (2)	No
36	1.4	Female	III	1884	No	CITA	2	I	Yes (1)	No
37	1.4	Male	IV	121,900	No	CITA/ITEC*	8	IV	No	No
38	0.9	Male	IV	51,000	No	CITA/ITEC	7	IV	No	No
39	0.8	Male	IV	65,000	No	CITA/ITEC	4	IV	No	No

*The case underwent transarterial chemotherapy with or without embolization.
†The case underwent liver resection before chemotherapy.
‡The case underwent auto-SCT twice with high-dose chemotherapy.

obstruction, gastric bleeding, PV obstruction, and refractory ascites. Five of the 23 patients (21.7%) who underwent hepaticojejunostomy developed biliary complications, as did 5 of the 16 patients (31.3%) who underwent duct-to-duct anastomosis did. Five of the 15 patients (33.3%) who had undergone hepatectomy before LDLT developed biliary complications, and 5 of the 24 cases (20.8%) without hepatectomy before LDLT also developed complications. Eight patients (20.5%) died during the follow-up period, although none of them lost their grafts because of surgical complications. In terms of toxicities related to chemotherapy, 3 patients among the long-term survivors developed mild renal dysfunction (cases 12, 34, and 39), and 1 patient

TABLE 2. Patient Characteristics at LDLT and Patient Outcomes

Case Number	Age at LDLT (Years)	AFP at LDLT (ng/mL)	Donor Age (Years)	Graft Type	Surgical Complications	Histopathological Type of HB	Histological Vascular Invasion (Site)	Chemotherapy	Tumor Recurrence (Interval After LDLT)	Outcomes (Follow-Up Period in Years)
1	10.6	23	38	LLS	No	Fetal	No	CPA	No	Alive (15.2)
2	0.8	4390	35	LLS	No	Macrotrabecular	Yes (HV)	CPA	Lung, skin (5 months)	Died (0.6)
3	7.5	54,700	37	LLS	No	Embryonal	Yes (PV, HV)	CBDCA + VP-16 + CPA*	Lung, graft, diaphragm, central nervous system (2.8 years)	Died (3.4)
4	2.6	5749	27	LLS	Biliary leakage, gastric bleeding	Embryonal	Yes (PV, HV)	CBDCA + VP-16	Lung (3 months)	Died (0.9)
5	4.6	12,924	30	LLS	No	Embryonal	Yes (PV)	CBDCA + THP-ADR	No	Alive (12.5)
6	11.0	383	47	LLS	No	Fetal	No	CBDCA + VP-16	No	Alive (11.8)
7	3.0	10	40	LLS	PV obstruction	Fetal	No	CITA	No	Alive (11.5)
8	5.3	37	29	LLS	Intra-abdominal bleeding	Combined	Yes (PV)	None	No	Alive (10.6)
9	6.9	1411	32	LL	Biliary stricture	Embryonal	Yes (PV)	None	No	Alive (9.2)
10	4.0	7040	25	LLS	Biliary stricture	Embryonal	Yes (PV, HV)	CITA	No	Alive (8.6)
11	5.3	4930	32	LLS	No	Embryonal	Yes (PV, HV)	CITA/ CPT-11	Lung, graft, PD (7 months)	Died (3.1)
12	12.4	113	40	LL	Biliary stricture	Fetal	Yes (PV, HV)	CBDCA + VP-16	No	Alive (7.6)
13	3.9	170,910	31	LLS	Bowel perforation	Embryonal	Yes (PV, HV)	CPT-11/ CPA	Lung, graft (1 month)	Died (0.5)
14	9.1	12	44	LLS	Biliary leakage	Fetal	Yes (PV)	CPT-11	No	Alive (7.4)
15	0.9	7008	41	Reduced LLS	Biliary stricture	Combined	Yes (PV)	CPT-11	No	Alive (6.6)
16	1.3	40	33	LLS	No	Fetal	No	CBDCA + THP-ADR	No	Alive (5.9)
17	4.7	1,175,690	29	LLS	No	Combined	Yes (PV, HV)	CPT-11	Lung (10 months)	Alive (6.5)
18	2.3	136,840	39	LLS	No	Unknown	No	None	No	Alive (6.1)
19	2.0	4264	32	LLS	Biliary stricture	Combined	Yes (PV)	CPT-11	No	Alive (6.1)
20	12.1	1471	44	LLS	Biliary stricture	Combined	No	CPT-11	No	Alive (6.0)

TABLE 2. *Continued*

Case Number	Age at LDLT (Years)	AFP at LDLT (ng/mL)	Donor Age (Years)	Graft Type	Surgical Complications	Histopathological Type of HB	Histological Vascular Invasion (Site)	Chemotherapy	Tumor Recurrence (Interval After LDLT)	Outcomes (Follow-Up Period in Years)
21	0.9	3155	23	LLS	Refractory ascites	Combined	No	None	No	Alive (5.9)
22	14.7	153	40	LL	Biliary stricture	Fetal	No	CPT-11	No	Alive (5.7)
23	3.6	93,000	34	LLS	Intra-abdominal bleeding	Embryonal	Yes (PV)	ITEC	Lung (4 months)	Alive (5.0)
24	7.5	616	31	LLS	No	Fetal	No	CPT-11	No	Alive (4.7)
25	1.1	1,061,480	29	LLS	Biliary stricture	Combined	Yes (PV)	CPT-11	Graft, PD (1.3 years)	Died (1.6)
26	2.5	1331	35	LLS	No	Mixed	No	None	No	Alive (4.6)
27	1.5	67,078	31	Reduced LLS	No	Combined	Yes (PV, HV)	ITEC/ CPT-11/CITA†	Diaphragm (3 months)	Died (0.9)
28	7.1	21,938	34	LLS	No	Macrotrabecular	Yes (PV)	CPT-11†	Graft, PD, systemic lymph nodes (1.1 years)	Died (1.3)
29	3.0	19,740	64	LL	No	Combined	Yes (PV, HV)	None	No	Alive (3.6)
30	3.7	617,900	25	LLS	No	Macrotrabecular	Yes (PV, HV)	CPT-11*	Lung, graft (2 months)	Alive (3.5)
31	1.7	50	38	LLS	Bowel obstruction	Fetal	No	CPT-11	No	Alive (2.8)
32	2.4	331	30	LLS	No	Macrotrabecular	Yes (PV, HV)	CPT-11	Lung (6 months)	Alive (2.6)
33	2.9	1919	45	LLS	No	Embryonal	Yes (PV)	CITA	No	Alive (2.4)
34	22.1	48	54	LL	Intra-abdominal bleeding	Fetal	No	CPT-11	No	Alive (2.2)
35	6.7	12,112	46	LLS	Intra-abdominal bleeding	Combined	No	CPT-11	No	Alive (2.0)
36	3.6	32	26	LL	No	Fetal	No	None	No	Alive (1.6)
37	2.0	1871	27	LLS	No	Combined	Yes (HV)	CPT-11	No	Alive (1.5)
38	1.4	278	30	LLS	No	Fetal	No	ITEC	No	Alive (1.4)
39	1.3	161,476	38	LLS	No	Fetal	Yes (PV, HV)	CITA	No	Alive (1.3)

*The case underwent auto-SCT with high-dose chemotherapy.
†The case underwent bone marrow transplantation.

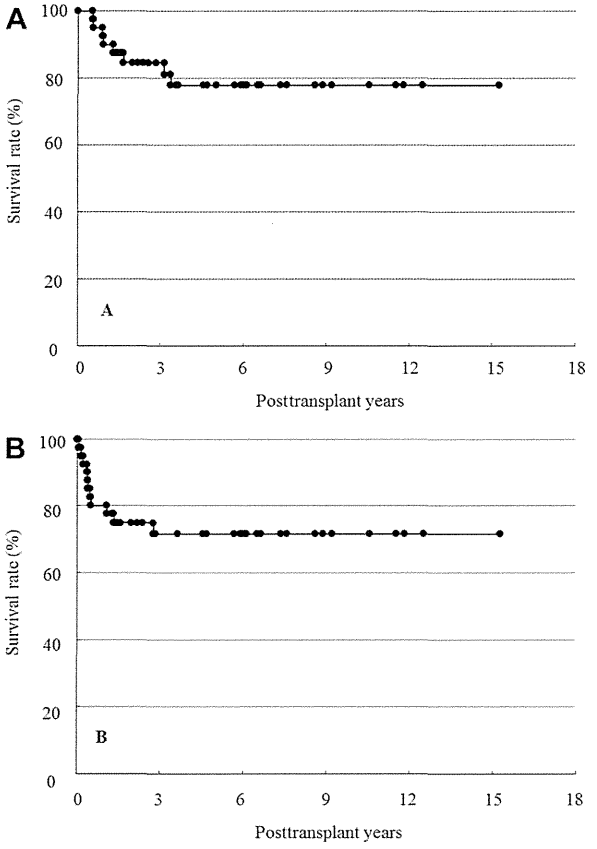

Figure 2. (A) Overall patient survival rate and (B) tumor recurrence-free survival rate.

developed mild auditory impairment (case 24). The overall survival rates were 84.3%, 77.3%, and 77.3% at 3, 5, and 10 years, respectively (Fig. 2A).

Histopathological Examination of the Explanted Liver

The histopathological type of HB in the explanted liver could not be determined in 1 case because of missing data; the rest were the fetal type in 13 cases (34.2%), the combined fetal and embryonal type in 11 cases (28.9%), the embryonal type in 9 cases (23.7%), the macrotrabecular type in 4 cases (10.5%), and a mixed epithelial and mesenchymal type in 1 case (2.6%). Twenty-four cases (61.5%) showed vascular invasion, including invasion into both the PVs and the hepatic veins (HVs) in 12 cases, the PVs in 10 cases, and the HVs in 2 cases.

Details of Tumor Recurrence After LDLT

Postoperative chemotherapy was performed for 32 cases (82.1%). CPT-11 was used as the initial post-transplant chemotherapy in 16 cases; this was followed by CITA in 5 cases, CBDCA and VP-16 in 4 cases, ITEC in 3 cases, CBDCA and THP-ADR in 2 cases, and cyclophosphamide in 2 cases. Postoperative chemotherapy was started at a median of 34 days (range = 0-203 days); this was dependent on the patient's postoperative condition.

Twelve cases (30.8%), all of whom received postoperative chemotherapy, showed tumor recurrence. The median interval between LDLT and the onset of tumor recurrence was 5.2 months (range = 1 month to 2.8 years). The most common site of tumor recurrence was the lung (9 cases), which was followed by the hepatic graft (6 cases), peritoneal dissemination (PD; 3 cases), the diaphragm (2 cases), the skin (1 case), multiple lymph nodes (1 case), and meningeal dissemination (1 case). Four of the 9 cases (44.4%) with lung metastasis underwent surgical resection, and 3 were alive without any tumor recurrence (cases 17, 23, and 32). Two of the 6 patients (33.3%) with tumor recurrence within the hepatic graft underwent surgical resection and radiofrequency ablation, respectively. Although the cases with tumor recurrence received additional systemic chemotherapy, including auto-SCT in 2 cases (cases 3 and 30) and bone marrow transplantation in 2 cases (cases 27 and 28), after they had undergone high-dose chemotherapy, 8 of the 12 cases (66.7%) died because of tumor recurrence, which included complications related to bone marrow transplantation as a direct cause of death (case 27); the median interval from the onset of tumor recurrence was 6 months, with the period ranging from 2 months to 2.6 years. The recurrence-free survival rates were 76.9%, 68.3%, and 68.3% at 1, 3, and 5 years, respectively (Fig. 2B).

Prognostic Factors for Tumor Recurrence After LDLT (Tables 3 and 4)

A univariate log-rank test for identifying the prognostic factors for tumor recurrence revealed that an AFP level at diagnosis $\geq$ 500,000 ng/mL, the presence of extrahepatic lesions before LDLT, a donor age $\leq$ 39 years, an AFP level at LDLT $\geq$ 4000 ng/mL, the histopathological type of HB, and histopathological vascular invasion were significantly associated with a higher incidence of tumor recurrence after LDLT. Although the AFP levels at diagnosis and LDLT were significant prognostic factors, the rate of the decline in the AFP level during the period from the diagnosis to LDLT did not reach statistical significance as a prognostic factor. The episodes of hepatectomy before LDLT (so-called rescue LDLT) were not a significant prognostic factor. The multivariate landmark analysis also showed that the independent risk factors for recurrence were a high AFP level at diagnosis (HR = 7.86, P = 0.010), the presence of extrahepatic lesion(s) before LDLT (HR = 3.82, P = 0.042), and a high AFP level at LDLT (HR = 9.19, P = 0.036).

DISCUSSION

Multicenter trials for HB, conducted by the Children's Oncology Group and the Childhood Liver Tumour Strategy Group of the International Society of

TABLE 3. Prognostic Factors for Recurrence Identified by the Univariate Analysis

Characteristic	n (%)	Recurrence-Free Survival Rate (%)		*P* Value*
		1 Year	3 Years	
Age at diagnosis				
<2 years old	18 (46.2)	77.8	71.8	0.75
≥2 years old	21 (53.8)	76.2	65.5	
Sex				
Male	28 (71.8)	75.0	66.3	0.82
Female	11 (28.2)	81.8	72.7	
PRETEXT stage				
IV	22 (56.4)	72.7	63.3	0.28†
III	15 (38.5)	80.0	80.0	
II	1 (2.6)	100.0	0.0	
I	1 (2.6)	100.0	N/A	
AFP at diagnosis				
<500,000 ng/mL	22 (56.4)	90.9	90.9	<0.01
≥500,000 ng/mL	17 (43.6)	58.8	41.2	
Extrahepatic lesion(s)				
Yes	13 (33.3)	53.8	46.2	0.01
No	26 (66.7)	88.9	76.8	
Factor‡				
V	1 (7.7)	0.0	N/A	0.02
P	3 (23.1)	33.3	N/A	
E	4 (30.8)	50.0	50.0	
R	2 (15.4)	50.0	N/A	
M	3 (23.1)	66.7	66.7	
Chemotherapy before LDLT				
Number of cycles				
≤6	22 (56.4)	72.7	63.3	0.35
≥7	17 (43.6)	88.2	74.1	
Initial protocol				
CITA	36 (94.7)	75.0	65.5	0.36
Others	2 (5.3)	100.0	100.0	
Transarterial chemoembolization				
Yes	10 (26.3)	70.0	60.0	0.44
No	28 (73.7)	78.6	70.2	
Stem cell transplantation				
Yes	9 (23.7)	77.8	77.8	0.52
No	29 (76.3)	75.9	63.9	
POST-TEXT stage				
IV	22 (62.9)	68.1	58.7	0.20†
III	11 (31.4)	81.8	81.8	
I	2 (5.7)	100.0	N/A	
Liver resection before LDLT				
Yes	15 (38.5)	86.7	78.0	0.24
No	24 (61.5)	70.8	62.2	
Number of resections				
≥2	7 (46.7)	85.7	85.7	0.73
1	8 (53.3)	87.5	72.9	
Period of resection				
Before chemotherapy	3 (20.0)	100.0	50.0	0.58
After chemotherapy	12 (80.0)	83.3	83.3	
Age at LDLT				
<3 years old	17 (43.6)	76.5	70.1	0.92
≥3 years old	22 (56.4)	77.3	67.1	
Interval: diagnosis to LDLT				
<1.5 years	28 (71.8)	71.4	64.1	0.26
≥1.5 years	11 (28.2)	90.9	77.9	
Donor age				
<39 years old	28 (71.8)	67.9	55.9	0.02
≥40 years old	11 (28.2)	100.0	100.0	

TABLE 3. *Continued*

Characteristic	n (%)	Recurrence-Free Survival Rate (%)		*P* Value*
		1 Year	3 Years	
Donor sex				
Male	20 (51.3)	70.0	70.0	0.90
Female	19 (48.7)	84.2	65.2	
Graft type				
LL	6 (15.4)	100.0	100.0	0.23
LLS	31 (79.5)	74.2	63.4	
Reduced LLS	2 (5.1)	50.0	50.0	
ABO-type compatibility				
Compatible	37 (94.9)	75.7	66.7	0.39
Incompatible	2 (5.1)	100.0	100.0	
IVC removal				
Yes (complete or partial)	6 (15.8)	83.3	83.3	0.49
No	32 (84.2)	75.0	64.6	
Immunosuppression at initiation phase				
CNI only	14 (35.9)	85.7	64.3	0.75
CNI with steroids	25 (64.1)	72.0	72.0	
AFP at LDLT				
<4000 ng/mL	20 (51.3)	95.0	95.0	< 0.01
≥4000 ng/mL	19 (48.7)	57.8	40.1	
AFP decline rate: diagnosis to LDLT				
Log-1 decline				
Yes	27 (69.2)	81.5	77.8	0.08
No	12 (30.8)	66.7	45.7	
Log-2 decline				
Yes	17 (43.6)	82.4	82.4	0.14
No	22 (56.4)	72.7	57.0	
ACR				
Yes	13 (33.3)	69.2	51.3	0.20
No	26 (66.7)	80.8	76.9	
Surgical complications				
Yes	18 (46.2)	83.3	77.8	0.33
No	21 (53.8)	71.4	59.3	
Histological type of HB				
Fetal type	13 (34.2)	100.0	100.0	< 0.01
Embryonal type	9 (23.7)	55.6	41.7	
Combined§	11 (28.9)	81.8	72.7	
Mixed‖	1 (2.6)	100.0	100.0	
Macrotrabecular	4 (10.5)	25.0	N/A	
Histological vascular invasion				
Yes	24 (61.5)	62.5	48.5	< 0.01
No	15 (38.5)	100.0	100.0	
Site of vascular invasion				
PV	10 (41.7)	90.0	70.0	0.18
HV	2 (8.3)	50.0	N/A	
Both PV and HV	12 (50.0)	41.7	31.3	
Chemotherapy after LDLT				
Yes	32 (82.1)	71.9	61.1	0.07
No	7 (17.9)	100.0	100.0	
Chemotherapy protocol				
CPT-11	16 (50.0)	75.0	62.5	0.97
Others	16 (50.0)	68.8	60.2	

*Log-rank test.
†Only compared between cases with PRETEXT stage III and cases with PRETEXT stage IV.
‡V indicates tumor invasion into the IVC and/or 3 HVs, P indicates tumor invasion into the portal trunk and/or bilateral main portal branches, E indicates tumor invasion into other adjacent organs or lymph node metastasis, R indicates tumor rupture, and M indicates metastasis.
§Combined fetal and embryonal type.
‖Mixed epithelial and mesenchymal type.

TABLE 4. Prognostic Factors for Recurrence Identified by the Multivariate Analysis

Pretransplant Factor	HR	95% CI	*P* Value
AFP at diagnosis			
<500,000 ng/mL	1		
≥500,000 ng/mL	7.86	1.62-38.06	<0.01
AFP at LDLT			
<4000 ng/mL	1		
≥4000 ng/mL	9.19	1.16-73.09	0.04
Extrahepatic lesion(s)			
No	1		
Yes	3.82	1.05-13.93	0.04

Paediatric Oncology (SIOPEL), continue to explore the best therapeutic strategies, and the overall survival rate has increased to nearly 80% in the most recent trials.[18,19] For further improvements of outcomes, the refined protocol provided by each multicenter trial has focused on the therapeutic strategy for high-risk HB, including guidelines for LT.[20] Long-term survival rates ranging from 55% to 100% have now been reported for multiple single-center series over the last 2 decades, which collectively show a median survival rate of approximately 80%.[6] The current study had a 77.3% overall patient survival rate and a 68.3% recurrence-free survival rate at 5 years with a median follow-up period of 4.6 years, and these were acceptable outcomes.

The current study was a retrospective review performed to analyze the outcomes of LDLT for HB, even though our own multicenter protocol (JPLT-1 and JPLT-2) was conducted in parallel as background, and it did not include the guidelines for LT. Furthermore, medical coverage of LT for HB was not approved until April 2008. For these reasons, the current study included cases with unresectable HB, which were treated with multiple sessions of chemotherapy with auto-SCT and/or multiple surgical resections before LDLT. Otte et al.[5] has demonstrated that survival with primary LT is significantly superior to survival with rescue LT according to data gathered from experienced transplant centers worldwide. Rescue LT is indicated for cases with incomplete tumor resection and/or intrahepatic recurrence after partial liver resection. It can be reasonably presumed that PRETEXT stage III or IV tumors are likely to be in close proximity to the main vessels, and this can lead to incomplete tumor resection. Even when the resection margins are macroscopically negative for a tumor in a specimen, microscopic residual tumors may be present at the resection line. Tumors that recur after liver resection with adequate chemotherapy may be a more aggressive type of tumor within the spectrum of behavior.[6,21] Therefore, primary LT can be recommended to prevent any attempt at liver resection when radical resection seems difficult.[5,22] On the other hand, a recent report by Lautz et al.[23] revealed excellent outcomes after aggressive resection in children with HB involving 3 or 4 sectors of the liver after neoadjuvant chemotherapy. They recommended careful consideration of all information available before one chooses primary LT or liver resection for cases with likely unresectable HB on a case-by-case basis because LT is not without morbidity and mortality. Furthermore, we have to heavily rely on living donors as an organ resource in our country, and avoiding LDLT for a patient who may have a chance to be cured by liver resection would, therefore, be preferable. The current study did not show inferior outcomes for rescue LT in comparison with primary LT, although the high proportion of cases undergoing liver resection without the option of primary LT at the same time should be taken into consideration. The current JPLT-3 protocol study, which includes surgical guidelines compatible with international validated guidelines (eg, SIOPEL[24]), is trying to draw a definite conclusion for this issue.

The current study showed that serum AFP levels at the time of diagnosis and at LDLT were significant prognostic factors related to tumor recurrence in both univariate and multivariate analyses despite the limitations associated with the retrospective nature of the analysis and the small sample size. A couple of reports have highlighted the possible negative influence of a very high AFP level on outcomes.[25,26] Because the clinical behavior, the presence of extrahepatic lesions before LDLT, the histopathological features of the tumor, the histopathological type, and histopathological vascular invasion were also significant prognostic factors related to tumor recurrence, the serum AFP level at diagnosis can predict outcomes after LDLT as an indicative parameter of the biological nature of the tumor. On the other hand, the serum AFP level at LDLT might be related to the quantitative burden of the residual tumor after pretransplant treatment because microscopic tumor dissemination can occur during the total hepatectomy procedure. Previous studies have revealed that patients with a good response to preoperative chemotherapy have better outcomes after LT in comparison with those with a poor response,[22,27] although the rate of decline in the AFP level during the period from diagnosis to LDLT did not reach statistical significance as a prognostic factor in the current study. This difference from the previous studies may be due to the pretransplant clinical course, which was affected by the various therapeutic modalities before LDLT. The serial changes in the AFP levels of the cases receiving chemotherapy only before LDLT can be differently interpreted from those of the cases with tumor recurrence after liver resections.

One-third of the patients in the current study had extrahepatic lesions before LDLT. Among them, the cases showing direct invasion into the IVC or the adjacent organs (stomach and transverse colon) at the time of LDLT developed tumor recurrence at a relatively early time point after LDLT. There is no doubt that LT is contraindicated for cases showing direct

tumor invasion at the time of LDLT. The cases with a macroscopic tumor thrombus within the PV at LDLT also had a high incidence of tumor recurrence after LDLT in the current study, as previous series similarly reported.[28] One of the 3 cases experienced a disappearance of the tumor thrombus after neoadjuvant chemotherapy and did not exhibit any tumor recurrence, and both of the other cases with remaining macroscopic tumor thrombi at the time of LDLT survived for relatively long periods after LDLT because the sites of recurrence could be managed by surgical resection. With respect to microscopic venous invasion, the existence of tumor invasion in the HVs might be a more significant risk factor for tumor recurrence than invasion in the PVs. The present data suggest that LT can be considered for cases with a macroscopic tumor thrombus within the PV, whereas those with macroscopic venous invasion in the major HVs and the IVC may be considered to have a relative contraindication for LT.

The presence of pulmonary metastasis at diagnosis still remains controversial with respect to indications for LT. It is obvious that LT should be considered for cases with pulmonary metastasis when the pulmonary lesions disappear after chemotherapy. One of the 3 cases in the current study experienced recurrence after LDLT, and the recurrent tumor developed at a site in the left pulmonary lobe similar to the site at which the pulmonary metastasis had been observed at diagnosis (case 32; Y. Inomata, Kumamoto University, written communication, 2013). Therefore, even when metastatic lesions radiologically disappear after chemotherapy, microscopic tumor foci may still remain. The questions remain whether pulmonary metastases can persist after chemotherapy, whether they can be surgically resected, and whether the patients should subsequently be eligible for LT. One patient in our series, who underwent the surgical resection of 4 pulmonary lesions before LDLT, did not show tumor recurrence for 3.5 years after LDLT. That case received high-dose chemotherapy with auto-SCT and then underwent LDLT (case 29; T. Yagi, Okayama University, personal communication, 2013). Because such pulmonary lesions are probably more chemotherapy-resistant, more aggressive chemotherapy with stem cell transplantation may represent an effective therapeutic option that can be given before LT. On the basis of the results of the SIOPEL-1 study, which revealed long-term recurrence-free survival for 4 of 5 patients (80%) with pulmonary metastases at the time of diagnosis, Otte et al.[5,29] suggested that LT might be considered for cases with pulmonary metastases with a paramount prerequisite of complete eradication by chemotherapy and/or surgical resection. This requires meticulous scrutiny of the lungs before LT by high-resolution radiological modalities.[6]

The management of patients after LT, including the immunosuppression regimen and chemotherapy, is also still controversial. Our series included 14 cases treated with a steroid-free regimen because of the presumption of a high risk of infections and tumor recurrence.[8] There were no significant differences among the patients treated with different types of immunosuppression in terms of tumor recurrence and infections, and the incidence of ACR was not higher than the standard incidence of ACR after LDLT.[30] Although detailed data, such as the target trough levels of immunosuppressants, were not obtained, the immunosuppression regimen could be considered to be standard.

A recent report from Wagner et al.[31] showed that rapamycin effectively inhibited HB growth in both in vitro and in vivo studies. The potential benefits of other types of immunosuppressants with antitumorigenic properties, such as rapamycin, require further evaluation. The use of postoperative chemotherapy remains an open debate. Seven cases in the present series did not undergo chemotherapy after LDLT, and they did not show any tumor recurrence. No specific characteristic related to the clinical and laboratory data before and after LDLT could be found, and the consideration about the necessity of postoperative chemotherapy was left to each center's discretion. We believe that postoperative chemotherapy should be considered for cases with extrahepatic lesions before LT, including macroscopic/microscopic vascular invasion, which was clearly defined as a significant prognostic factor for tumor recurrence. The selection of the chemotherapy regimen after LT should be based on the effectiveness for the tumor and the side effects of the preoperative chemotherapy regimen. A recent report revealed that CPT-11 had significant antitumor activity and acceptable toxicity in patients with relapsed HB.[32] Half of the cases in our series were treated with CPT-11 as postoperative chemotherapy, although this agent did not show any significant superiority in terms of recurrence-free survival. Further prospective studies of postoperative chemotherapy are needed.

In conclusion, a nationwide survey of the outcomes of LDLT for HB in Japan, in which 39 patients were enrolled, showed excellent results. A multivariate analysis revealed that the independent risk factors for recurrence were a high AFP level at diagnosis (500,000 ng/mL), the presence of extrahepatic lesions before LDLT, and a high AFP level at LDLT (4000 ng/mL). With respect to extrahepatic lesions before LDLT, the presence of macroscopic venous invasion and viable extrahepatic lesions not amenable to surgical excision should be a contraindication for LT. However, the current retrospective study included cases with different backgrounds with respect to therapeutic decisions before and after LDLT for a relatively long study period. Further investigations through the nationwide management protocol (conducted by the JPLT) may clarify the precise indications for LT as a treatment for HB.

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ORIGINAL ARTICLE

Isolated intestinal neuronal dysplasia Type B (IND-B) in Japan: results from a nationwide survey

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Abstract
Purpose Intestinal neuronal dysplasia Type B (IND-B) has been proposed to be an allied disorder of Hirschsprung's disease (ADHD). The original histological criteria included hyperganglionosis, giant ganglia, ectopic ganglion cells and an increased AChE activity in the lamina propria. The criteria for IND-B have been gradually revised. The present diagnostic criteria are [1] more than 20 % of the submucosal ganglia contain nine or more ganglion cells and [2] the patient is older than 1 year. To clarify the current status of IND-B in Japan, a nationwide retrospective cohort study was performed.
Methods Questionnaires were sent to 161 major institutes of pediatric surgery and gastroenterology in Japan.
Results A total of 355 cases of ADHD were collected, including 18 cases of IND-B (5 %). Based on original criteria, 13 out of 18 cases were diagnosed as IND-B. However, only four cases met the current criteria. Three of the four patients (75 %) required pull-through operation. All of the patients exhibited giant ganglia and ganglioneuromatosis-like hyperplasia of the myenteric plexus.
Conclusions IND-B cases matching the current criteria are thought to be quite rare and they are associated with marked hyperplasia of the myenteric plexus. "True" IND-B is a rare and intractable disease.

Keywords Intestinal neuronal dysplasia · Allied disorders of Hirschsprung's disease · Variant Hirschsprung's disease · Giant ganglia · Ganglioneuromatosis

Introduction

"Allied disorders of Hirschsprung's disease" (ADHD) have been understood to be conditions that clinically resemble Hirschsprung's disease (HD), despite the presence of ganglion cells in the terminal rectum [1]. The patients with Hirschsprung's disease generally present in the newborn

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Table 1 Classification of variant Hirschsprung's disease (Puri P. 1997)

1. Intestinal neuronal dysplasia	Abnormal ganglia
2. Hypoganglionosis	
3. Immature ganglia	
4. Absence of argyrophil plexus	
5. Internal anal sphincter achalasia	Normal ganglia
6. Smooth muscle cell abnormalities	
7. Perinuclear vacuolation	
8. MMIHS	

Table 2 Classification of the allied disorders of Hirschsprung's disease (Japanese study group for ADHD)

Morphologically abnormal ganglia (HE or AchE)
Immaturity of ganglia
Hypoganglionosis (oligoganglionosis)
Congenital hypoganglionosis
Acquired hypoganglionosis
IND
Morphologically normal ganglia (HE or AchE)
CIIP (idiopathic CIP or CPO)
MMIHS
Segmental dilatation of the intestine
IASA

period with delayed passage of meconium and abdominal distention, or as young children with severe chronic constipation. The patients with ADHD show similar symptoms and signs to HD, however, they can be distinguished from HD patients by the pathological findings. The term "Pseudo HD" was proposed to describe these disorders by Ravitch in 1958 [2]. They encountered patients referred for the treatment of megacolon in whom the difficulty lay elsewhere than in the congenital absence of ganglion cells of the myenteric plexuses in a segment of the rectum or the colon and rectum. Ehrenpreis summarized these conditions as "HD and allied disorders" in the "Seminar on Pseudo-Hirschsprung's Disease and Related Disorders" [3]. The main point was that the various disease patterns were essentially determined by their underlying pathology He classified ADHD into two categories based on the histological findings: those with abnormalities of ganglion cells and those without abnormalities of ganglion cells. In 1997, Puri proposed that "Variant Hirschsprung's disease" (VHD) is a more appropriate description, and that VHD includes eight disorders: intestinal neuronal dysplasia (IND), intestinal ganglioneuromatosis, hypoganglionosis (HG), immature ganglia, the absence of the argyrophil plexus, internal anal sphincter achalasia (IASA), smooth muscle cell abnormalities, perinuclear vacuolation and megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) [4]. The former four disorders are considered to be associated with abnormal ganglia, while the latter four disorders are considered to occur in patients with normal ganglia (Table 1). We therefore decided to use the term ADHD, because Holschneider and Puri have used this term in their book Hirschsprung's Disease and Allied Disorders [1].

In Japan, E. Okamoto and A. Toyosaka used the term "Pseudo-Hirschsprung's disease" in the Japanese literature [5]. They defined this term as a congenital, non-mechanical obstruction of the intestine with the presence of intramural ganglion cells in the terminal rectum. He classified the diseases into two categories based on the histological findings of abnormal or normal ganglia. The abnormal ganglia were found in patients with immaturity of the ganglia (IG), HG, hypogenesis, and IND, while those with normal ganglia included patients with chronic idiopathic intestinal pseudoobstruction (CIIP) [6] and MMIHS.

According to the literature and the previous Japanese version of Okamoto's classification, ADHD was classified into two categories depending on the pathological findings in terms of the acetylcholinesterase (AchE) staining and/or HE staining: (a) Abnormal ganglia, including IG, HG and IND, (b) Normal ganglia, including cases with MMIHS, segmental dilatation (SD), IASA and CIIP (Table 2).

IND was first described by Meier-Ruge in 1971 in children who presented with clinical symptoms resembling HD [7]. The histological findings included hyperplasia of the submucosal and myenteric plexuses, and an increased acetylcholinesterase (AchE) activity in the lamina propria. Fadda et al. classified the diseases into two types, A and B, where Type A is very rare (<5 %), and the majority of IND cases are classified as Type B [8]. IND Type A is characterized by congenital aplasia or hypoplasia of the sympathetic innervation. Patients with IND A typically present in the neonatal period with abnormal distension, bowel obstruction and episodes of diarrhea with hemorrhagic stools, while IND Type B is characterized by hyperplasia of the parasympathetic plexus and the symptoms of patients with IND B are resembling Hirschsprung's disease. Recently, isolated IND-B has been considered to be almost synonymous with IND. There have been several reports describing the histology of IND, and as a result, the typical histological findings have thus been established as hyperganglionosis, giant ganglia, ectopic ganglion cells and increased AChE activity in the lamina propria and around the submucosal vessels [9]. These criteria are considered to be "original criteria". Two disease types, an isolated form and association with Hirschsprung's disease, were reported shortly after their report [10]. At present, IND-B refers to "isolated" IND-B.

Table 3 Summary of 4 cases of "true" IND-B

	Year of birth	Sex	Birth weight	Onset	Symptom	Anomaly	Plain X-P	Ba enema	Manometry	Rectal biopsy (age)	Operation (age)	Histology of operative specimen	Current condition	Medication
1	1999	M	3,328	Neonate	Constipation, failure of thrive	None	Not available	No caliber change, megarectum	Reflex (−)	Giant ganglia (3y 5 m)	Soave (3y 7m)	Giant ganglia, ganglioneuromatosis-like hyperplasia of Auerbach	Mild constipation, occational suppository	None
2	2001	M	3,262	Infant	Distention, failure of thrive	Cardiac (PS), CFC syndrome	Dilatation of intestine	Caliber change, megacolon	Not done	Giant ganglia (10 m)	TAEPT (1y 7m)	Not available	Defecation from colostomy occasional irrigation from stoma	Necessary
											Colon biopsy (2y 7m)	Giant ganglia, ganglioneuromatosis-like hyperplasia of Auerbach		
											Transverse colostomy (5y)	Not available		
											left hemicolectomy (7y 6m)	giant ganglia, ganglioneuromatosis-like hyperplasia of Auerbach		
											Partial colectomy (8y 4m)	Giant ganglia, ganglioneuromatosis-like hyperplasia of Auerbach		
											Re-colostomy at right colon (9y 10m)	Not available		
3	2005	F	3,260	Neonate	Distention, vomiting	None	Dilatation of intestine	Megacolon	Reflex (−)	Giant ganglia (1y6 m) Giant ganglia (4y)	Sphincter myectomy (2y)	Ganglioneuromatosis-like hyperplasia of Auerbach	Annual enemas (every 2 days)	Necessary
											Colostomy at left colon (2y 6 m)	Giant ganglia, ganglioneuromatosis-like hyperplasia of Auerbach		
											Laparoscopic pull-through (5y)	Giant ganglia, ganglioneuromatosis-like hyperplasia of Auerbach		
4	2011	F	3,430	Neonate	Distention, vomiting	Minor (club foot)	Dilatation of intestine	Megacolon	Reflex (+)	Giant ganglia (6m) Giant ganglia (2y 9m)	Not done		Annual enemas (every 2–3 days)	None

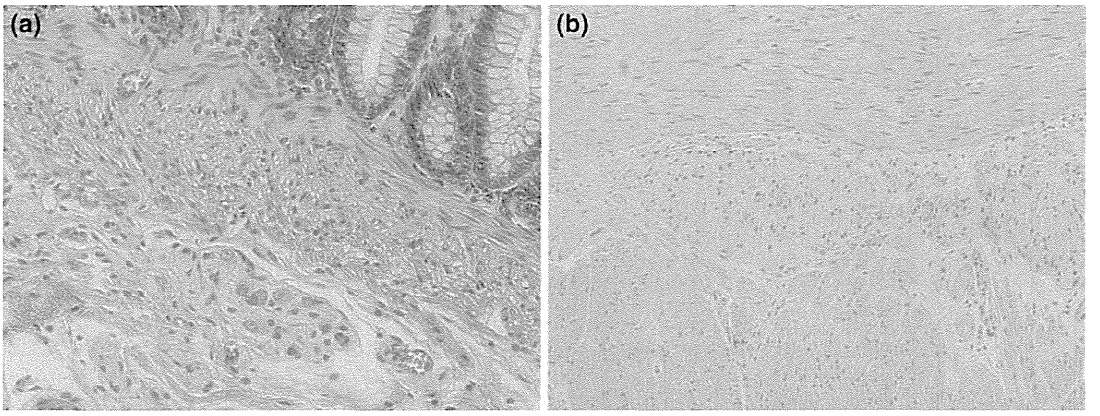

Fig. 1 The histological findings of a specimen of left colon at 3y 7m in Case 1 (HE). **a** Giant ganglia in the submucosa. **b** The myenteric plexus shows a ganglioneuromatosis-like hyperplasia

The incidence of IND-B varies depending on the institute and by country. There has also been debate about whether IND-B is a real disease. Furthermore, the criteria for IND-B have been gradually revised by the Meier-Ruge group. The diagnostic criteria used at present are: (1) more than 20 % of submucosal ganglia contain nine or more ganglion cells (with at least 25 ganglia evaluated) and (2) the patient must be older than 1 year, because giant ganglia may be misinterpreted in infants due to the fact that immature ganglia and AchE activity in the lamina propria mucosae have been shown to be age-dependent phenomena that disappear upon the maturation of the submucosal plexus [1, 11, 12].

To clarify the current status of IND-B in Japan, a nationwide 10-year, retrospective cohort study was performed.

Patients and methods

As a nationwide retrospective cohort study, supported by the Ministry of Health and Welfare, Japan, preliminary questionnaires requesting the number of cases of ADHD seen from January 2000 to December 2009 and the criteria used at each institute, were sent to the 161 major institutes of pediatric surgery or pediatric gastroenterology, representing the core members of the Japanese Society of Pediatric Surgeons, the Japanese Society of Pediatric Nutrition, Gastroenterology and Hepatology and the Japanese Study Group of Pediatric Constipation. Therefore, almost all institutes which were treating ADHD were considered to be included. The number of patients, including the definite and suspected cases, based on the tentative classification of ADHD (Table 2), was requested. We also asked about the criteria used to diagnose these diseases by each institute to be answered as free descriptions. The criteria for "definitive" or "suspected" depended on each institute.

As a preliminary study, the questionnaires asking about the number of cases of ADHD, including IND-B, and the criteria for each disorder were sent to the 161 major institutes of pediatric surgery and pediatric gastroenterology in Japan, to collect information about the cases of ADHD treated during the 10 years from 2001 and 2010. The criteria for IND-B used for the survey were the original histological criteria [9], as described previously. Subsequently, a case report form with a questionnaire for each case was sent and collected as part of a detailed survey.

This study was performed according to the Ethical Guidelines for Clinical Research published by the Ministry of Health, Labor and Welfare of Japan on July 30, 2003. This study was approved by the ethics committee for clinical research of Kyushu University Hospital (No. 24-163).

Results

Responses were obtained from 157 out of the 161 institutes (98 %). Ninety-five institutes (61 %) had treated a total of 355 ADHD patients. These included 18 IND-B (5 %) patients. According to the answers to the questionnaires, 69 out of the 95 (73 %) institutes which experienced cases of ADHD had some diagnostic criteria; 34 out of the 69 (50 %) had criteria for IND-B. The major criteria for IND-B at these institutes were as follows: increased AchE-positive fibers in the lamina propria in 17/34 institutes (50 %), ectopic ganglion cells in 14/34 institutes (41 %), giant ganglia (>5 ganglion cells per plexus, based on original criteria) in 13/34 institutes (38 %), severe constipation or rectal dysmotility in 9/34 institutes (26 %) and hyperganglionosis in 6/34 institutes (18 %).

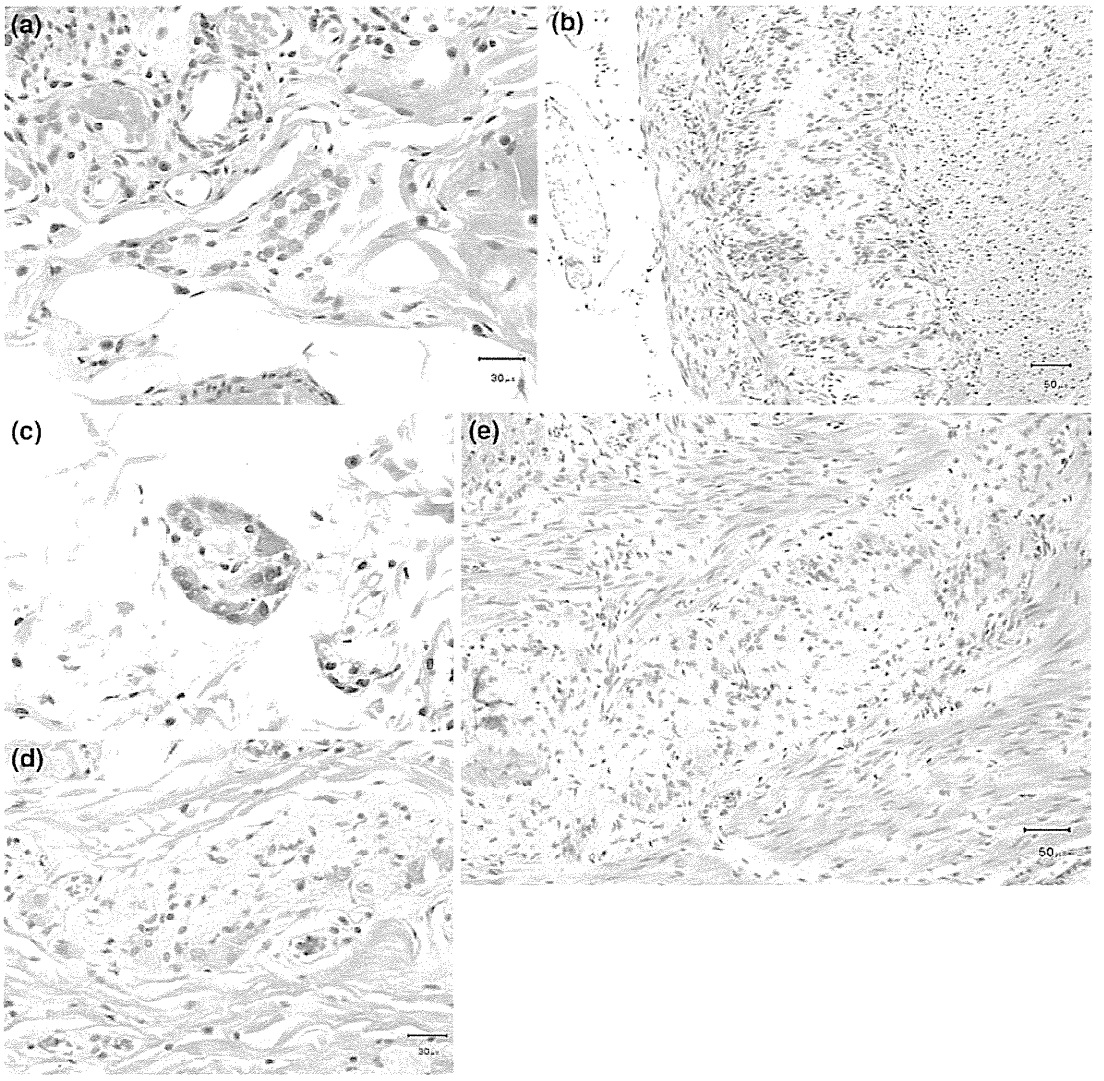

Fig. 2 The histological findings of a full-thickness specimen of left colon in Case 2 at 7 years (HE). **a** Giant ganglia in the submucosa. **b** Prominent ganglioneuromatosis-like hyperplasia of myenteric plexus. The histological findings of a full-thickness specimen of left colon in Case 2 at 8 years (HE). **c** Giant ganglia in the submucosa. **d** Prominent ganglioneuromatosis-like hyperplasia of submucosal plexus. **e** Prominent ganglioneuromatosis-like hyperplasia of myenteric plexus

Out of 18 case report forms sent as part of a secondary survey, 15 were subsequently collected. Two of these cases were excluded because they were a duplicates, or because there were no histological criteria for IND-B. Finally, 13 cases of IND-B were included in this survey. The clinical symptoms were abdominal distention (12/13), vomiting (6/13) and constipation (6/13). Histological examinations showed giant ganglia (7/13), increased AchE fibers (9/13) and ectopic ganglia (5/13). Surgical procedures (enterostomy and/or pull-through) were performed in seven cases (54 %).

These 13 cases were then reevaluated according to the most recently used diagnostic criteria [1, 11, 12], which stipulate that [1] more than 20 % of at least 25 submucosal ganglia examined contain nine or more ganglion cells and [2] the patient must be older than 1 year. As a result, only four cases met the current criteria (Table 3). All of them were initially treated by conservative therapy, including enemas and laxatives. One of the four cases (Case 4) has been successfully treated conservatively. One case (Case 3) underwent anal sphincter myectomy at age 2 years, but it was not effective. Finally, three out of the four cases (Cases 1, 2 and 3) required bowel resection and pull-through [13]. Currently, two out of the three patients that were surgically treated still required enemas or suppository, and the remaining one required a permanent colostomy. Two cases (Cases 2 and 3) still require medical treatment.

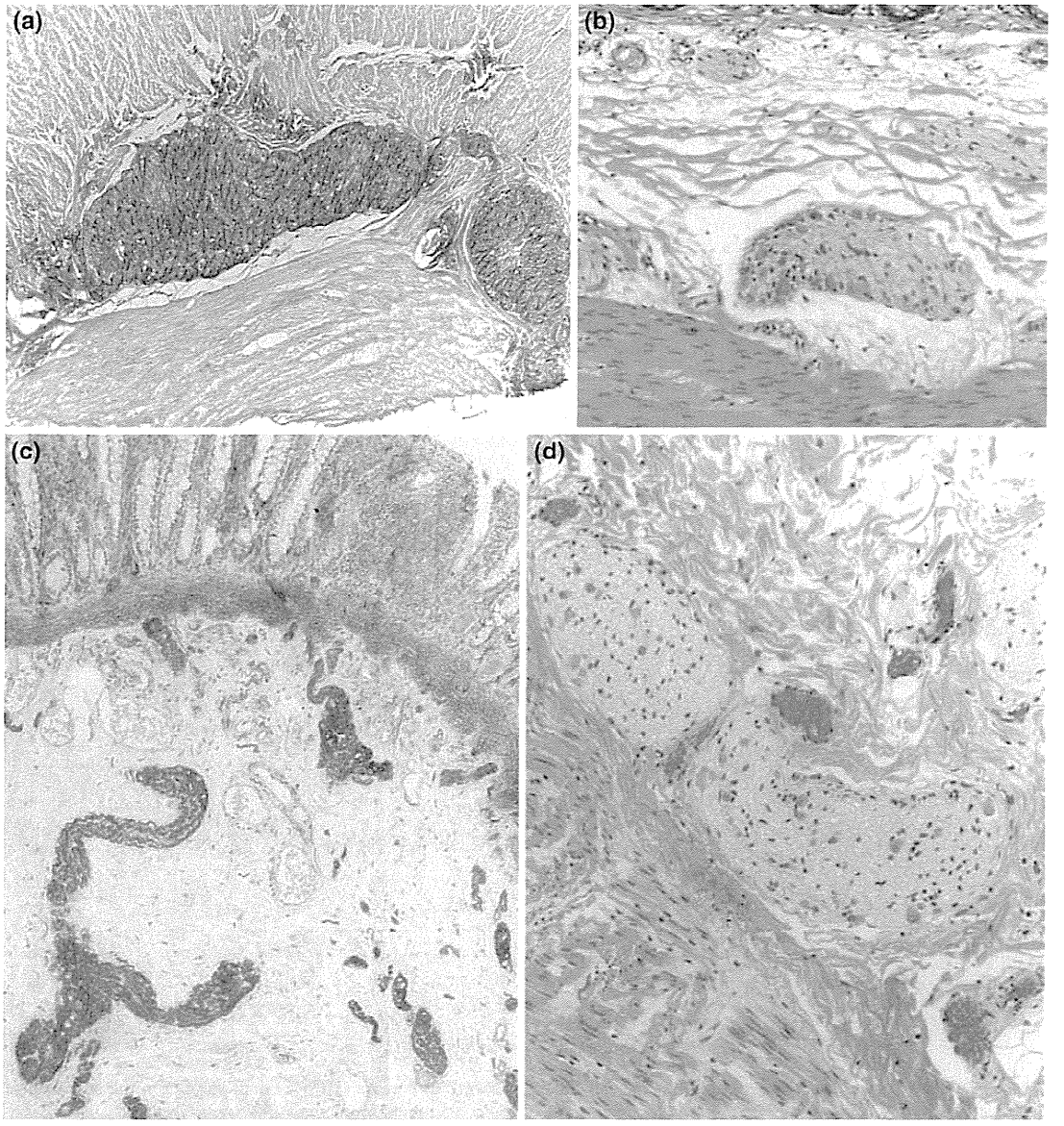

Fig. 3 The histological findings of Case 3. **a** AchE staining showed a markedly hyperplastic Auerbach's plexus in the rectal myectomy specimen. **b** HE staining revealed giant ganglia with ganglioneuromatosis hyperplasia of Meissner's plexus in the left colon. **c** AchE staining at 4y showed markedly hyperplastic ganglioneuromatosis-like hyperplasia of submucosal plexus in the right colon. **d** HE staining at 5y showed giant ganglia with a hyperplastic ganglioneuromatosis-like appearance in the submucosa in the transverse colon

All of the patients showed giant ganglia, including more than nine ganglion cells, in the submucosa after they were 1 year old; full-thickness specimens from the three surgically treated cases showed marked hyperplasia of myenteric as well as submucosal plexus (Figs. 1, 2, 3, 4). In these three cases, hyperplastic plexus showed an increase in all elements of the plexus, including Schwann cells, neurons, and ganglion cells. These findings are mimicking ganglioneuromatosis of the gastrointestinal tract-associated MEN-2b [14].

Discussion

IND was first reported by Meier-Ruge in 1971 [7]. Shortly afterward, Puri et al. reported a case of IND associated with HD [10]. Fadda et al. classified the disease into two types, A and B, with the majority of IND cases being classified as Type B [8]. Recently, isolated IND-B has been considered to be almost synonymous with IND. Therefore, we collected the cases of isolated IND-B as "IND" cases in our Japanese survey.