lengthened. Five of the patients required fresh frozen plasma to supplement their coagulation factors after vitamin K failed to normalize the prothrombin time.

Hypergalactosaemia is usually accompanied by cataracts and should be treated with lactose-free formula as soon as it is detected. The screen-negative patients were free from hypergalactosaemia at newborn screening, but developed it subsequently (Tamamori et al 2004; Tazawa et al 2004). Galactose concentration should be checked whenever a citrin deficiency is suspected.

Hypoglycaemia was present in 18 patients. We speculate that this is caused by a disturbance of gluconeogenesis, because the aspartate–glutamate carrier (citrin) provides substrates for gluconeogenesis as a part of the pathway for the conversion of amino acids to glucose (Hachisu et al 2005; Saheki and Kobayashi 2002; Tamamori et al 2002). Hypoglycaemia may be a common feature in patients with citrin deficiency.

Most of the patients with NICCD were initially diagnosed with neonatal hepatitis syndrome before the DNA diagnosis, and were thus treated according to protocols for this condition (Balistreri 1985). MCT-enriched formula, fat-soluble vitamins, ursodeoxycholic acid, and phenobarbital were widely used. Lactose-free formula was used for the patients with hypergalactosaemia. When Naito and colleagues (Naito et al 2002) performed lactose challenge tests on an infant with NICCD, the first challenge at 56 days led to a worsening in the liver function tests and a return to hypergalactosaemia, whereas the re-challenge at 152 days did not worsen the laboratory findings. These data suggest that lactose may be toxic to patients with NICCD while cholestasis persists. In our study, however, 18 patients improved without the use of special milk formula.

Two of the screen-negative patients suffered liver failure and underwent liver transplantation before their first birthdays. The first patient (case 1 in Tamamori et al 2002) initially presented at 2 months of age because of poor weight gain and jaundice. Thereafter she was followed carefully, but her liver function tests worsened from age 6 months and liver failure ensued. She underwent living-related donor liver transplantation at age 10 months. The second patient was referred to hospital at age 3 months because of jaundice. His liver function progressively worsened and he finally developed hepatic dysfunction. He underwent living-related donor liver transplantation at 5 months (unpublished data). Interestingly, the provisional diagnosis was tyrosinaemia type I in both cases, even though succinylacetone was not detected in their urine. Patients with NICCD should be followed closely to identify the rare cases with a poor prognosis. One patient who recovered from NICCD before his first birthday developed CTLN2 and required liver transplantation at age 16 (Tomomasa et al 2001). It is important to collect more clinical data on patients with NICCD and CTLN2 to elucidate genetic and environmental factors which determine the age of onset, course and prognosis in patients with CTLN2.

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Original Article

Histological findings in the livers of patients with neonatal intrahepatic cholestasis caused by citrin deficiency

Akihiko Kimura,[1] Masayoshi Kage,[2] Ikuo Nagata,[3] Sotaro Mushiake,[4] Toshihiro Ohura,[5] Yusaku Tazawa,[6] Shunichi Maisawa,[7] Takeshi Tomomasa,[8] Daiki Abukawa,[5] Yoshiyuki Okano,[9] Ryo Sumazaki,[10] Masaki Takayanagi,[11] Akiko Tamamori,[12] Tohru Yorifuji,[13] Yasuhiko Yamato,[1] Kohji Maeda,[1] Masami Matsushita,[1] Toyojiro Matsuishi,[1] Ken Tanikawa,[2] Keiko Kobayashi[14] and Takeyori Saheki[14]

[1]Department of Pediatrics and Child Health, [2]Department of Pathology, Kurume University School of Medicine, Kurume, [3]Department of Pediatrics, Faculty of Medicine, Tottori University, Yonago, [4]Department of Pediatrics, Faculty of Medicine, Osaka University, Osaka, [5]Department of Pediatrics, Tohoku University School of Medicine, Sendai, [6]Department of Pediatrics, South Miyagi Medical Center, Shibata, [7]Department of Pediatrics, Morioka Children's Hospital, Morioka, [8]Department of Pediatrics, Gunma University School of Medicine, Maebashi, [9]Department of Pediatrics, Osaka City University Graduate School of Medicine, Osaka, [10]Department of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, [11]Department of Pediatrics, Chiba Children's Hospital, Chiba, [12]Department of Pediatrics, Fujiidera City Hospital, Fujiidera, [13]Department of Pediatrics, Kyoto University, Kyoto, and [14]Department of Molecular Metabolism and Biochemical Genetics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Aim: To characterize the histological features of the livers of patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), we studied specimens from 30 patients diagnosed with NICCD by genetically analyzing the *SLC25A13* gene.

Methods: Liver biopsy specimens were subjected to hematoxylin–eosin, Azan, and Berlin-blue staining.

Results: Most specimens showed varying degrees of fibrosis. The degree of inflammation varied among the specimens, with half showing moderate or severe inflammatory changes. Fat deposition in hepatocytes was observed in almost all of the specimens, and severe fatty liver was noted in 20 (67%) of them. There was a mixture of two types of hepatocytes with macrovesicular or microvesicular fat droplets, and cholestasis was observed at a rate of 77%. Hemosiderin deposition, mostly mild and localized in periportal hepatocytes and macrophages in portal areas, was observed in 57% of the specimens.

Conclusion: A combination of mixed macrovesicular and microvesicular fatty hepatocytes and the above-described findings, such as fatty liver, cholestasis, necroinflammatory reaction and iron deposition, are almost never observed in other liver diseases in infants and adults. We believe that NICCD is a disease with characteristic hepatopathological features.

Key words: citrin, citrullinemia, fatty liver, fibrosis, neonatal intrahepatic cholestasis caused by citrin deficiency, *SLC25A13*.

INTRODUCTION

SAHEKI *ET AL.* reported that the enzyme abnormalities of citrullinemia can be classified as qualitative, type I and type III, or quantitative, type II.[1,2] The first, the classical form (CTLN1), is found in most patients with neonatal/infantile-onset citrullinemia, and was first described by McMurray *et al.*[3] In CTLN1, the enzyme defect is found in all tissues in which argininosuccinate synthetase (ASS) is expressed.[1,2,4] The second form, type II citrullinemia (CTLN2) is an adult- or late childhood-onset liver disease characterized by a liver-specific defect in ASS, and most of these patients have a fatty liver.[5] This enzyme abnormality is caused by a deficiency in citrin, a calcium-binding

Correspondence: Professor Masayoshi Kage, Department of Pathology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. Email: masakage@med.kurume-u.ac.jp
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mitochondrial solute carrier protein which is encoded by the *SLC25A13* gene.[6]

Recently, several cases of *SLC25A13* mutations have been reported in early infancy with cholestatic liver disease.[7–13] Yamaguchi *et al.*[14] designated these findings as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). Citrin deficiency causes two age-dependent phenotypes, CTLN2 in adults and NICCD in infants.[15] Most NICCD patients showed hypoproteinemia, galactosemia, multiple aminoacidemia including citrullinemia, methionemia and tyrosinemia, cholestasis, and have a fatty liver.[7–13] Only a few papers have described the pathology of the NICCD[8,9,11,13] or CTLN2[5] liver.

Therefore, the present study was designed to clarify the histological findings of the NICCD liver.

METHODS

Patients

WE STUDIED THE liver histological findings of 30 patients aged 2.9 ± 1.7 months with a range of 1–7 months consisting of 17 men and 13 women who had been diagnosed with NICCD with *SLC25A13* mutations by genetic analysis including five patients who were documented in previous reports.[7–11] Moreover, mutations in *SLC25A13* were detected in both alleles of 29 patients and in a single allele of one patient. Mutation detection and DNA diagnosis of the *SLC25A13* gene were performed as previously described ([6,14,16] and T. Saheki et al., 2006, unpublished data). Moreover, we examined biochemical data within 1 week before or after liver biopsy for 30 patients with NICCD.

Methods

Liver biopsy specimens from 30 patients diagnosed with NICCD were subjected to hematoxylin–eosin, Azan, and Berlin-blue staining. The grading of fibrosis and inflammation was based on Ludwig's Classification with slight modifications (Table 1).[17] The other histopathological features were graded as none, mild, moderate and severe, and scored as 0, 1, 2 and 3, respectively.

Grading was independently performed by three pathologists, and the grade for each specimen was determined by consensus between two or three of them.

Relationship between age and histological findings

To clarify the relationship between age and the histological findings, the cases were divided into three groups according to their ages: group A, less than 2 months old; group B, 3–4 months old; and group C, more than 5 months old. The average of the grading score of the histological findings for each group was then obtained.

Table 1 Histological classification of liver biopsy

Stage of fibrosis	
Stage 0	No portal fibrosis
Stage 1	Mild to moderate fibrous expansion of portal tract
Stage 2	Bridging fibrosis between portal tracts without lobular distortion
Stage 3	Bridging fibrosis between portal tracts with lobular distortion
Stage 4	Liver cirrhosis
Grade of inflammation	
Grade 0	None (0)
Grade 1	Mild (1–3)
Grade 2	Moderate (4–6)
Grade 3	Severe (≥7)

Parentheses indicate scores derived by Ludwig's scoring system.

Statistical analysis

The data regarding the relationship between age and histological findings were analyzed using the Mantel–Haenszel linear trend test. *P*-values less than 0.05 were regarded as statistically significant.

RESULTS

Patients

THE PROGNOSIS OF almost NICCD patients at 1 year of age was fairly well. However, some NICCD patients had developed progressive liver failure by then. For example, two patients developed liver failure by 6 months (patient 28) and 7 months (patient 30)[10] of age and one patient (patient 9) developed behavioral aberrations, which included shouting and episodes of violence, by 16 years of age.[9,18] Two patients, one with liver failure[10] and one with mental derangement,[9,18] received a living-related liver transplant. Therefore, the outcomes of the NICCD patients were not always favorable. We obtained four sets of follow-up liver biopsy specimens from patients 8, 9, 13 and 18 (data not shown).

From the clinical laboratory data, serum levels of citrulline, α-fetoprotein, ferritin and pancreatic secretory trypsin inhibitor (PSTI) were noted to have generally increased (Table 2). We also detected high serum levels of total and direct bilirubin, aspartate (AST) and/or alanine aminotransferase (ALT), total bile acids and

Table 2 Biochemical data on liver biopsy in the 30 patients with neonatal intrahepatic cholestasis caused by citrin deficiency

Patient No.	1	2	3	4	5	6	7	8	9	10	11
Age (months)/sex	1/M	1/M	1/M	1/M	1/F	1/F	2/M	2/M	2/M	2/M	2/M
Total/direct bilirubin (mg/dL)	9.0/3.4	12.6/2.6	3.3/2.2	10.4/5.8	5.6/1.9	3.3/0.7	6.2/3.8	9.9/5.4	7.6/3.3	6.6/2.6	3.6/1.6
AST/ALT (IU/L)	96/38	31/20	115/61	121/24	62/41	43/21	112/28	109/50	41/20	100/30	190/53
Total bile acids (μM)	250	120	513	298	210	52	323	331	n.d.	240	212
γ-GTP (IU/L)	206	142	131	251	186	148	142	408	130	n.d.	125
Total cholesterol (mg/dL)	212	195	n.d.	181	161	158	175	206	133	n.d.	196
Total protein/albumin (g/dL)	4.9/3.2	3.9/2.6	5.3/4.0	4.5/3.0	5.1/3.5	4.4/3.3	4.7/2.6	–/–	3.6/1.9	–/–	4.7/2.8
Citrulline (nmol/mL)	4.3	n.d.	85.0	n.d.	40.5	149.0	74.3	12.6	n.d.	117.0	211.0
α-Fetoprotein (ng/mL)	n.d.	n.d.	n.d.	200 700	n.d.	n.d.	n.d.	n.d.	29 600	n.d.	n.d.
PSTI (ng/mL)	n.d.	n.d.	n.d.	91.0	n.d.	40.0	24.0	n.d.	n.d.	n.d.	110.0
Ferritin (ng/mL)	447	n.d.	n.d.	2656	n.d.	117	502	1830	n.d.	n.d.	n.d.
Prothrombin activity (%)	75	26	93	55	37	88	70	37	9	n.d.	76
Mutation type	V/XIX	I/II	I/I	I/II	II/II	I/V	II/V	II/V	II/II	I/V	I/V

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2/M	2/F	2/F	2/F	2/F	3/M	3/M	3/M	3/F	3/F	4/M	4/M	4/F	4/F	5/M
10.2/3.9	11.1/3.6	13.0/8.5	6.9/2.7	5.3/2.4	6.1/3.5	6.0/3.8	9.6/2.7	8.8/3.2	12.0/2.6	5.1/2.5	6.7/3.7	5.4/3.5	6.2/2.4	15.0/10.1
106/22	86/23	133/45	78/25	74/44	98/36	232/48	85/44	95/39	75/19	95/90	295/105	208/100	83/24	146/66
240	320	172	290	143	302	269	205	389	157	283	172	253	127	355
213	132	78	209	160	124	249	n.d.	149	198	145	270	132	90	129
n.d.	n.d.	204	232	n.d.	194	n.d.	140	223	256	128	169	n.d.	n.d.	n.d.
4.9/3.7	4.0/3.5	3.8/2.6	4.1/2.7	n.d.	5.3/3.9	n.d.	5.7/3.8	5.1/3.1	4.8/3.0	4.2/3.5	4.8/3.1	5.5/3.5	n.d.	4.0/2.8
242.0	478.0	581.0	n.d.	291.7	839.1	208.0	n.d.	32.2	392.0	675.0	524.0	27.5	28.4	5.8
n.d.	n.d.	87 000	n.d.	91 940	n.d.	n.d.	n.d.	n.d.	n.d.	75 300	n.d.	n.d.	n.d.	10 578
n.d.	24.0	n.d.	n.d.	57.0	n.d.	n.d.	n.d.	62.0	12.9	12.5	n.d.	n.d.	n.d.	188.0
743	n.d.	775	n.d.	1651	n.d.	n.d.	n.d.	n.d.	200	n.d.	n.d.	503	n.d.	n.d.
87	n.d.	n.d.	25	51	43	n.d.	66	50	75	29	39	69	15	15
IV/IV	II/II	II/V	II/II	II/II	II/III	I/I	II/II	VI/VI	I/II	I/–	I/II	VIII/X	IV/VI	II/V

27	28	29	30	Mean ± SD	Range	Normal range
5/F	6/M	6/F	7/F			
5.8/3.4	5.5/3.9	6.2/2.0	5.9/2.9	7.6 ± 3.0/3.6 ± 2.0 (n = 30)	3.3–15.0/0.7–10.1	0.2–1.1/0.0–0.4
260/169	123/87	127/38	191/67	120.3 ± 63.7/49.2 ± 33.3 (n = 30)	31–295/20–169	6–40/5–40
213	n.d.	150	168	241.3 ± 96.1 (n = 28)	52–513	5–25
67	149	65	292	168.6 ± 75.0 (n = 28)	65–408	5–32
n.d.	148	n.d.	168	183.1 ± 34.8 (n = 19)	133–256	130–220
6.4/4.7	4.5/3.0	4.6/2.7	6.0/3.2	4.7 ± 0.7/3.2 ± 0.6 (n = 25)	3.6–6.4/1.9–4.7	6.5–8.3/3.7–5.2
48.2	11.0	41.3	86.8	179.1 ± 199.2 (n = 25)	4.3–291.7	17–43
n.d.	11 000	329 000	207 000	115 790.9 ± 108 111.0 (n = 9)	11 000–329 000	<10 000
n.d.	n.d.	21.9	n.d.	58.5 ± 53.6 (n = 11)	12.5–188.0	22–46
n.d.	n.d.	n.d.	197	874.6 ± 816.3 (n = 11)	117–2656	12–80
88	9	41	29	51.3 ± 26.0 (n = 25)	9–93	70–140
I/II	I/II	I/II	I/II			

AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; PSTI, pancreatic secretory trypsin inhibiter; M, male, F, female; n.d., not done; I, 851del4; II, IVS11 + 1G > A; III, 1638ins23; IV, S225X; V, IVS13 + 1G > A; VI, 1800ins1; VIII, E601X; X, IVS6 + 5G > A; XIXIVS16ins3kb; –, unknown; SD, standard deviation.

γ-glutamyl transpeptidase. Prothrombin activity, total protein and albumin were decreased. The mutation types were 851del4/IVS11 + 1G > A throughout most of late infancy, being more than 5 months of age in patients 27, 28, 29 and 30.

Histological findings

Histological findings of the 30 patients are shown in Table 3. The results of the fibrosis staging and inflammation grading are shown in Figure 1.

Fibrosis

Most specimens showed varying degrees of fibrosis; 35% of all specimens were classified as stage 0, while stages 1 and 2 together accounted for 50%. However, there was a wide spectrum of fibrosis: more advanced liver lesions with distorted lobular architecture (stage 3) (Fig. 2) and cirrhosis were observed in four and one specimens, respectively. One patient with cirrhosis developed hepatic failure. Therefore, this patient underwent a living-related liver transplant. One patient with cirrhosis developed at 10 months of age.[10]

Inflammatory reaction

The degree of inflammation varied with the specimens, where half showed moderate or severe inflammatory changes. Inflammatory cell infiltration in the portal tracts and piecemeal necrosis were observed (Fig. 3). Inflammatory cells present in the portal tracts were predominantly lymphocytes. Focal necrosis and acidophilic bodies in the parenchyma were seen in 23 (77%) and 12 (40%) specimens, respectively. The sinusoids showed the proliferation of mononuclear cells with scarce neutrophils and the activation of Kupffer cells.

Fat deposition in hepatocytes

Fat deposition in hepatocytes was observed in all specimens except one and severe fatty liver was noted for 20 (67%) specimens (Fig. 4a). Fat droplets deposited in the cytoplasm of hepatocytes varied in size, and fat-laden hepatocytes were classified as those with macrovesicular fat droplets, those with foamy, microvesicular fat droplets, and those with mixed macrovesicular and microvesicular fat droplets. Hepatocytes with microvesicular fat droplets had a centrally located nucleus. In 80% of 29 specimens with fat deposition including all 20 specimens which showed severe fatty livers, there was a mixture of macro- and microvesicular fat droplets (Fig. 4b,c). Macrovesicular and microvesicular fatty liver alone accounted for three (10%) and one (4%) specimens, respectively. A moderate and severe fatty liver with an inflammatory reaction and lipogranuloma were diagnosed as steatohepatitis, which accounted for 60% of the patients. The histopathological findings in this disease were different from those in non-alcoholic steatohepatitis. The clinical features of one patient who had no fat deposition in hepatocytes did not differ from that of other patients with such fat deposition.

Cholestasis

Cholestasis was observed in 77% of the specimens and was severe in 57%. The acinar arrangement of hepatocytes was prominent in specimens with severe cholestasis (Fig. 5) and multinucleated giant cell transformation was found in one case (Fig. 6).

Hemosiderin deposition

Hemosiderin deposition, mostly mild and localized in periportal hepatocytes and macrophages in portal areas (Fig. 4b), was observed in 57% of the specimens.

A combination of all five features, fatty liver, inflammatory cell infiltration, fibrosis, cholestasis and hemosiderin deposition was observed in the same liver biopsy specimen in 12 (40%) of the total specimens.

Relationship between the age and the histological findings

The mean score of each histological finding in each of groups A, B and C are summarized in Table 4. The degree of fibrosis, necroinflammatory reaction such as focal necrosis and acidophilic bodies, acinar arrangement of hepatocytes, cholestasis and steatohepatitis of infants more than 3 months old (groups B and C) were more accentuated than those of the early infants of group A. Conversely, hemosiderosis and extramedullary hematopoiesis in groups B and C were less pronounced than in group A. The staging score of fibrosis, grade of inflammation and steatohepatitis were significantly higher in the older than in the younger group in order of group A, B and C.

Histological findings of follow-up biopsy

Follow-up biopsies were conducted for patients 8, 9, 13 and 18, and the findings were as follows: patients 8, 9 and 13 showed histological deterioration of cholestasis and fatty change. Of note, patient 9 underwent a liver transplant at the age of 16 years because of hepatic failure. The findings for the explant liver were

Table 3 Histological findings of liver biopsy in the 30 patients with neonatal intrahepatic cholestasis caused by citrin deficiency

Patient no.	1	2	3	4	5	6	7	8	9	10
Stage of fibrosis	0	0	1	0	0	0	0	0	3	2
Grade of inflammation	1	2	2	1	1	1	2	1	1	1
Focal necrosis[a]	1	1	2	0	0	0	1	0	0	1
Acidophilic body[b]	0	1	0	2	0	1	0	1	0	0
Acinar arrangement[c]	0	1	3	3	0	1	0	1	2	1
Cholestasis[d]	0	3	3	3	1	0	1	2	3	1
Degree of fat deposit[e]	1	3	3	3	3	3	2	3	3	3
Type of fat deposit[f]	1	3	0	3	3	3	1	3	0	0
Steatohepatitis[g]	0	1	1	1	0	1	1	1	0	2
Hemosiderosis[h]	0	2	1	2	0	0	1	2	0	2
Extramedullary hematopoiesis[i]	0	2	0	3	2	1	0	2	0	0
Patient no.	11	12	13	14	15	16	17	18	19	20
Stage of fibrosis	0	2	2	1	0	0	3	2	1	1
Grade of inflammation	1	1	1	2	1	2	2	2	3	1
Focal necrosis	1	0	1	1	1	2	1	1	3	0
Acidophilic body	1	0	0	1	0	0	1	0	0	0
Acinar arrangement	2	0	0	2	2	1	1	1	2	1
Cholestasis	3	0	0	3	3	2	2	2	3	3
Degree of fat deposit	3	0	2	2	3	2	3	3	2	3
Type of fat deposit	3	0	2	3	3	3	3	3	3	3
Steatohepatitis	2	0	0	1	1	1	1	1	2	1
Hemosiderosis	2	0	1	0	2	1	1	0	2	1
Extramedullary hematopoiesis	0	0	0	3	2	0	1	0	0	0
Patient no.	21	22	23	24	25	26	27	28	29	30
Stage of fibrosis	2	2	0	2	2	3	1	3	3	4
Grade of inflammation	3	2	1	2	3	2	1	2	3	3
Focal necrosis	1	2	1	1	3	1	1	1	2	1
Acidophilic body	1	2	0	1	1	1	0	0	0	2
Acinar arrangement	3	2	0	2	2	1	2	1	3	2
Cholestasis	3	3	0	3	0	3	3	3	3	3
Degree of fat deposit	3	3	3	3	1	3	2	3	3	3
Type of fat deposit	3	3	3	3	1	3	3	3	3	3
Steatohepatitis	0	3	2	1	0	2	1	3	3	3
Hemosiderosis	3	1	1	1	0	1	1	0	0	0
Extramedullary hematopoiesis	1	0	1	1	2	0	0	0	1	0

[a]Focal necrosis was graded from 0–3 based on the number of counts per 10 fields at a magnification of ×40. A score of denotes 0 is none, 1 denotes 1–2; 2 denotes up to 4, and 3 denotes >4.
[b]Acidophilic bodies were counted and graded from 0–3, as similar to that for focal necrosis.
[c]The acinar arrangements of the hepatocytes were graded 0–3. A rating of 0 denotes none, 1 denotes involvement up to 30% of the hepatocytes, 2 denotes 30–60%, and 3 denotes >60%.
[d]Cholestasis was graded from 0–3. A score of 0 denotes none, 1 denotes cholestasis without a bile plug, 2 denotes scattered bile plugs, and 3 denotes frequent bile plugs.
[e]The degree of fat deposition in hepatocytes was graded from 0–3 based on the percentage of hepatocytes in the biopsy involved. A rating of 0 denotes none; 1 denotes up to 30%, 2 denotes 30–60%, and 3 denote >60%.
[f]The type of fat deposit was classified as being between 0–3. A score of 0 denotes no fatty change, 1 denotes predominantly macrovesicular fat droplets, 2 denotes predominantly microvesicular fat droplets, and 3 denotes mixed microvesicular and macrovesicular fat droplets.
[g]Steatohepatitis was graded from 0–3, where 0 denotes none, 1 denotes steatosis involving up to 60% and intra-acinar inflammation with no or mild portal inflammation, 2 denotes steatosis (>66%) with both acinar and portal inflammation, and 3 denotes panacinar steatosis with intra-acinar inflammation and piecemeal necrosis.
[h]Hepatocellular iron was graded between 0–3, where 0 denotes none, 1 denotes localized deposition in the portal and /or periportal area; 2 denotes iron deposition involving up to 60% of the parenchyma, and 3 denotes >60%.
[i]Extramedullary hematopoiesis was graded between 0–3, similar to that for focal necrosis.

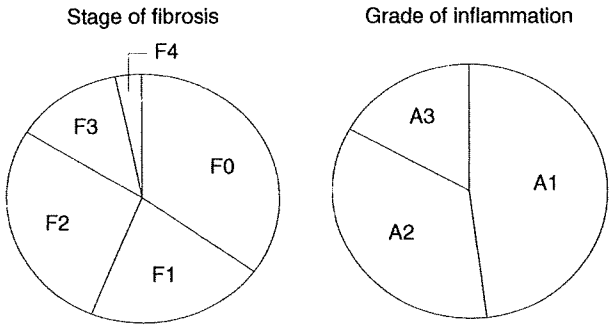

Figure 1 Results of fibrosis and the grade of necroinflammation.

more pronounced than those of the biopsy. Patient 8 showed progression of fibrosis from stage 1–3 and more pronounced portal inflammation. In contrast, patient 18 showed marked improvement of every

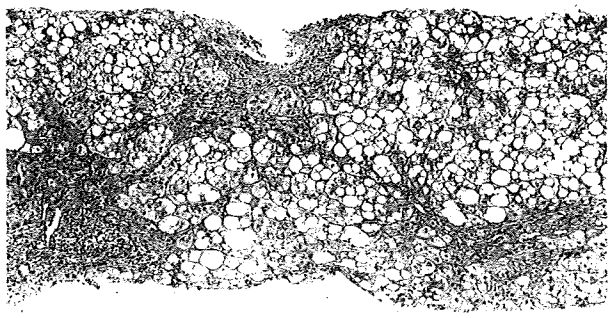

Figure 2 Severe fatty liver with distorted lobular architecture due to extensive fibrosis in stage 3 with portal inflammation (hematoxylin–eosin, original magnification ×50).

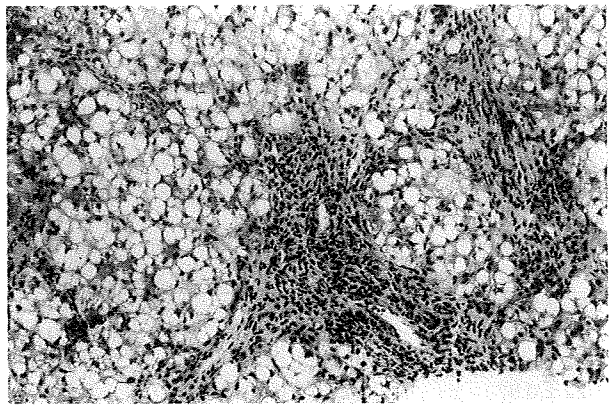

Figure 3 Fatty liver with moderate inflammatory cell infiltration in the portal tract and parenchyma, which is indicative of steatohepatitis (hematoxylin–eosin, original magnification ×100).

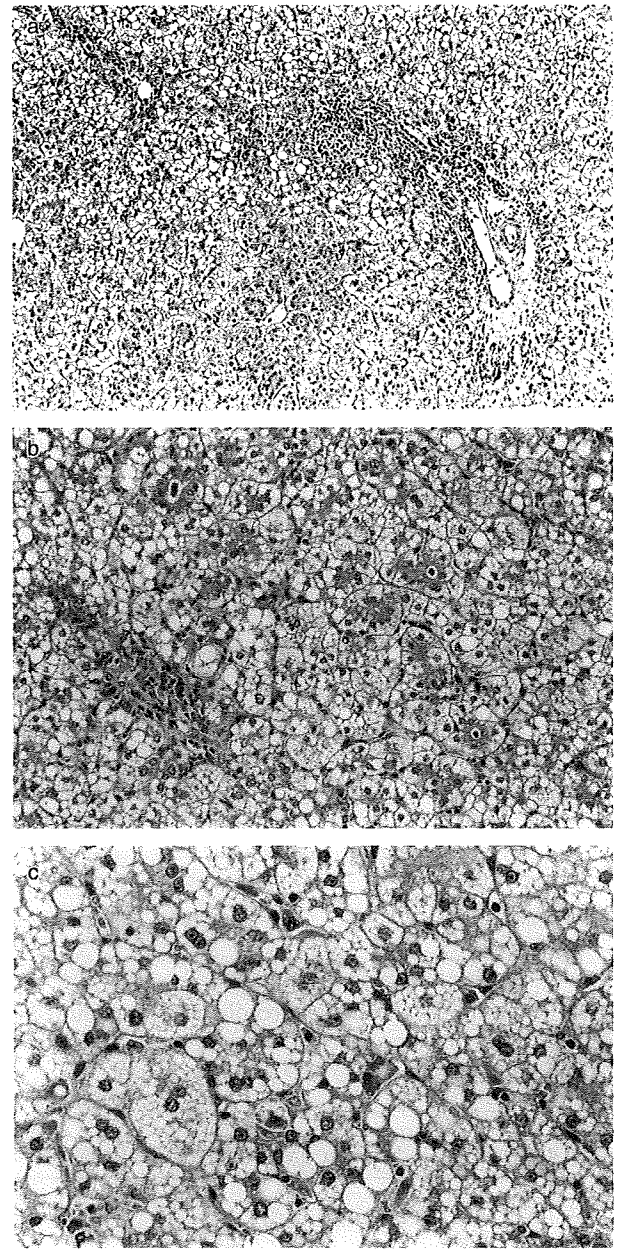

Figure 4 (a) Severe fatty liver with cholestasis. The portal tracts show mild inflammatory cell infiltration (hematoxylin–eosin [HE], original magnification ×50). (b) Pseudo-acinor transformation with bile plugs is observed. Hemosiderin-laden macrophages are present in a portal tract (HE, original magnification ×100). (c) Macro- and microvesicular-type fatty droplets. Some of the swollen hepatocytes have a foamy appearance and their cytoplasm packed with micro-fat droplets. Kupffer cells are activated (HE, original magnification ×200).

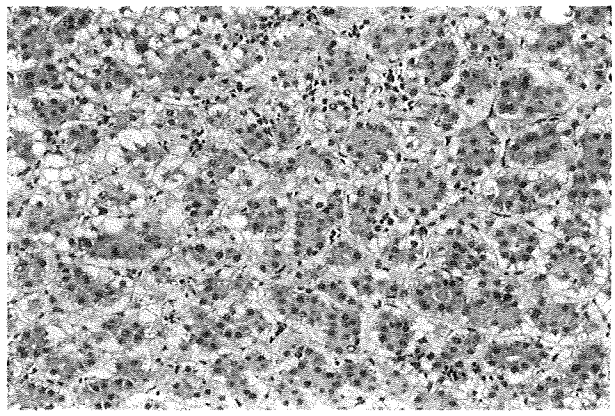

Figure 5 Striking pseudo-acinor transformation of the hepatic cords containing bile plugs. Small fatty droplets are present at the periphery of hepatocytes arranged in an acinar fashion (hematoxylin–eosin, original magnification ×100).

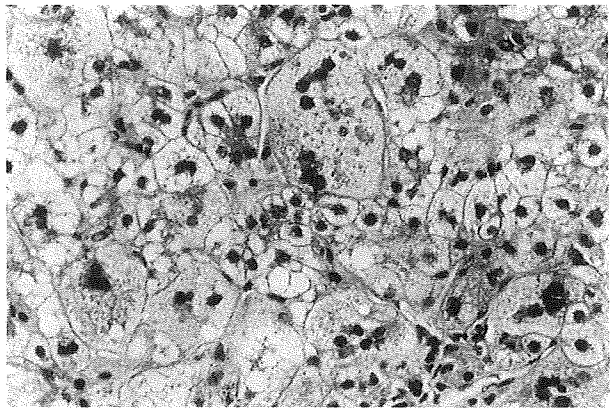

Figure 6 Giant cell hepatitis and cholestasis. Multinucleate giant cells contain several nuclei (hematoxylin–eosin, original magnification ×200).

histological finding, including decreased portal fibrosis and inflammation.

DISCUSSION

THE CAUSE OF liver dysfunctions such as fatty liver, hypoglycemia and galactosemia in this disease is as follows.[15] Citrin deficiency blocks the malate aspartate shuttle, which may increase the ratio of cytosolic nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide ($NADH/NAD^+$), which in turn is associated with the inhibition of glycolysis and makes reduced alcohol metabolism. This may be why CTLN2 patients dislike carbohydrates and cannot drink alcohol, and why alcohol consumption often results in psychiatric symptoms. An increased $NADH/NAD^+$ ratio is also characteristic of the inhibition of gluconeogenesis involving reduced substrates.[19] This, together with the reduction in alanine metabolization to urea and glucose due to citrin deficiency may cause hypoglycemia in NICCD patients. Although NICCD patients suffer from galactosemia, which sometimes even leads to the development of cataracts, no abnormalities in the enzymes involved in galactose metabolism have been found.[20] Because uridine diphosphate-glucose epimerase which requires NAD as a cofactor is strongly inhibited by NADH,[21] galactosemia in NICCD may represent a high NADH level in the cytosol of the liver.

From the biochemical data of this study, 50% of the high level of total bilirubin was associated with direct bilirubin, but it was not always dominant. The levels of AST were increased to more than twice the levels of ALT. Low levels of total protein, albumin and prothrombin

Table 4 Relationship between age and histological changes

Pathological findings	Group A ($n = 16$) <2 months	Group B ($n = 9$) 3–4 months	Group C ($n = 5$) >5 months	*P*-value
Stage of fibrosis	0.69 ± 1.01	1.67 ± 0.87	2.80 ± 1.10	$P = 0.001$
Grade of inflammation	1.31 ± 0.48	2.11 ± 0.78	2.20 ± 0.84	$P = 0.004$
Focal necrosis	0.75 ± 0.68	1.44 ± 1.01	1.20 ± 0.45	$P = 0.063$
Acidophilic body	0.44 ± 0.63	0.67 ± 0.71	0.60 ± 0.89	$P = 0.523$
Acinar arrangement	1.19 ± 1.05	1.56 ± 0.88	1.80 ± 0.84	$P = 0.172$
Cholestasis	1.75 ± 1.29	2.11 ± 1.27	3.00 ± 0.00	$P = 0.059$
Degree of fat deposit	2.44 ± 0.89	2.67 ± 0.71	2.80 ± 0.45	$P = 0.333$
Steatohepatitis	0.81 ± 0.66	1.22 ± 0.97	2.40 ± 0.89	$P = 0.008$
Hemosiderosis	1.00 ± 0.89	1.11 ± 0.93	0.40 ± 0.55	$P = 0.356$
Extramedullary hematopoiesis	0.94 ± 1.18	0.67 ± 0.71	0.20 ± 0.45	$P = 0.297$

The data are expressed as means ± standard deviation.
P-values are by the Mantel–Haenszel linear trend test.

activity and high levels of citrulline, α-fetoprotein, ferritin and PSTI were observed as previously described in NICCD patients.[6–13] However, 11 patients showed high levels of ferritin, which were not observed in previous reports on NICCD patients. Therefore, the pediatric hepatologist should suspect NICCD when a neonatal cholestatic patient has high levels of AST of more than twice the ALT value, citrulline, α-fetoprotein and ferritin, and low levels of total protein and prothrombin activity.

The histological findings in this study such as a fatty liver, cholestasis, necroinflammatory reaction and iron deposition are not pathognomonic findings because they occur in various liver diseases.[22] However, the combination of mixed macrovesicular and microvesicular fatty hepatocytes and these histological findings are almost never observed in other liver diseases in infants and adults. Microvesicular fatty deposition was found in NICCD, this type of fatty change is a characteristic feature of Reye syndrome[23] and other rare conditions.[22] However, the histogenesis of the microvesicular fatty deposition in NICCD is unclear. It might reflect the acute impairment of β-oxidation of fatty acid due to mitochondrial dysfunction as in Reye syndrome.

Although our series of NICCD patients shared common liver histological findings as described above, there seemed a tendency that late infants of group C had more advanced fibrosis and more accentuated inflammation than those of early infants of group A. The duration of illness may be an aggravating factor of the progression of the disease in some cases. There was no difference between the liver histological findings and mutation type. Interestingly, however, the mutation type of patients with severe fibrosis who were 6 and 7 months of age was 851del4/IVS11 + 1G > A. Because evidence for this relationship between the mutation type and the progression of fibrosis is not clear, further investigation is needed. Moreover, in the follow-up liver biopsy patients, we observed improvements in their liver histological findings as the liver dysfunction was ameliorated. Therefore, we speculate that the correlations between the stage of the liver histological findings and the biochemical test data exist.

This study found that NICCD is a disease with characteristic hepatopathological features. If NICCD is suspected in the presence of cholestasis during infancy, a liver biopsy should be performed to screen for liver diseases. We believe that a liver biopsy is of high diagnostic value for NICCD, and is useful for accurately assessing inflammation and the degree of the progression of fibrosis.

Although we were not able to elucidate the natural history of the disease, we previously found that despite a benign course in the majority of the patients, it leads to the development of liver cirrhosis in some patients with CTLN2.[5,10] This suggests that it involves the risk of progressive fibrosis and eventually leads to the development of cirrhosis. This possibility is suggested by the above histopathological findings characteristic of NICCD in the patients who progressed to stage 3 chronic hepatitis and cirrhosis. Although the process responsible for the progression of liver lesions is not clear, some patients with steatohepatitis including nonalcoholic steatohepatitis (NASH) progress to cirrhosis.[24] In this study, steatohepatitis was found in 60% of the specimens. It is likely that, in NICCD, steatohepatitis repeatedly deteriorates, persists and progresses.

In conclusion, if NICCD is suspected in the presence of cholestasis during infancy, a liver biopsy should be performed to screen for other liver diseases. NICCD is a disease with characteristic hepatopathological features, such as the combination of mixed macrovesicular and microvesicular fatty hepatocytes, cholestasis, necroinflammatory reaction and iron deposition. Therefore, it is possible to diagnose NICCD based on histological liver findings in most cases. However, when cirrhosis with fat deposition is detected in hepatocytes in liver specimens, the patient should be carefully observed, because the prognosis of NICCD patients is not always fair, with some developing progressive liver failure by 1 year of age. Finally, we could not infer the development of CTLN2 from the histological findings of the patients with NICCD who were examined in this study.

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低蛋白血症を伴う新生児肝炎—脂肪肝・肝ヘモジデローシスの合併

秋田大学医学部小児科[1]，東北大学医学部小児科[2]，八戸赤十字病院小児科[3]
もりおかこども病院小児科[4]，東北大学加齢医学研究所発達病態[5]
田澤　雄作[1]　虻川　大樹[2]　小宅　泰郎[3]
西宮　藤彦[1]　米沢　俊一[4]　相川純一郎[2]
大浦　敏博[2]　高田　五郎[1]　今野　多助[5]

キーワード：新生児肝炎，低蛋白血症，脂肪肝，肝ヘモジデローシス

要　旨

新生児肝炎は新生児・乳児早期に閉塞性黄疸を示す病因不明の疾患であり，その合併症は多様であるが，低蛋白血症は稀である．我々は過去10年間に低蛋白血症を合併した3例の新生児肝炎を経験したが，このほか生化学的に，ガラクトース血症，高アミノ酸血症，ビタミンK欠乏，高フェリチン血症の合併を認め，全例が肝組織学的に大脂肪滴型脂肪肝，肝ヘモジデローシス，門脈域線維化の所見を示した．入院後，特殊ミルクへの変更により全例速やかに改善・治癒した．一例で施行された1歳時肝生検所見は，軽度の門脈域線維化のほか，異常所見は認めなかった．本症の病因としては，鉄負荷・低栄養が考えられる．

緒　言

新生児肝炎の病因は不明であるが，多様な疾患が含まれていると推定され[1)2)]，最近では，胆汁酸代謝異常の報告が認められる[3)4)]．新生児肝炎の合併症は多様であるが[5)]，低蛋白血症の報告は稀であり，予報的に記載されているに過ぎない[6)7)]．一方，新生児期に閉塞性黄疸を示す疾患中，脂肪肝を示す疾患の代表としては，多様な代謝性疾患があげられる[8)~12)]．しかし肝組織学的に脂肪肝・ヘモジデローシスを合併する疾患の報告は，主として代謝性疾患に限られている[11)~13)]．我々は，新生児肝炎の臨床像を示し，低蛋白血症を合併，脂肪肝，肝ヘモジデローシスを示す特異な肝組織学的所見を示した3症例を経験したので報告する．

症例・方法

新生児肝炎の診断は，以下の厚生省特定疾患「難治性肝炎」「肝内胆汁うっ滞」調査研究班（昭和50年）の定義を満たす症例とした：1）新生児期に発症したと考えられるもので，多くは生後2カ月以内に発見された肝内胆汁うっ滞で，顕性黄疸は1カ月以上持続し，多くは6カ月以内に消褪する．灰白色便（または淡黄色便）および濃黄色尿を伴う．2）組織学的には巨細胞性肝炎の像を見ることが多い．3）ただし，尿路感染症，敗血症，梅毒，その他の全身性感染症あるいは全身性代謝性疾患などに伴った二次性のものを除く．

我々は過去10年間（1986～1995）に60例の新生児肝炎（検査時日齢15～80）を経験したが，血清総蛋白値は3.2g/100mlから7.0g/100mlの範囲に分布し（5.8±0.7g/100ml），57例は4.9g/100ml以上の値を示した．我々は，4.1g/100ml以下の低蛋白血症を示した3症例の臨床像，生化学的検査所見，肝組織像所見，臨床経過について検討したので報告する．

［症例1］生後53日の女子，在胎38週，2,700gにて出生する．血族結婚歴は認めず，流産歴も認めない．第1子は健康な3歳男子，患児は第2子であり，新生児先天性代謝異常スクリーニングの結果は正常であった．母親には出生前8週間の鉄剤内服歴がある．生後母乳栄養が開始されたが，入院数週前から人工乳が追加されている．生後1カ月頃から黄疸が増強し，黄疸・肝機能異常・体重増加不良（入院時体重，3,800g）を主訴として東北大学小児科に入院する．入院時理学的所見としては，黄疸，灰白色便，濃黄色尿，肝腫大（右

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秋田大学医学部小児科　　田澤　雄作

表1　一般血液生化学的検査成績ほか

		症例1	症例2	症例3
総ビリルビン	mg/dl	6.9	9.1	12.6
直接ビリルビン	mg/dl	3.1	5.6	2.6
総胆汁酸	μmol/L	209	172	120
GOT	IU/L	78	86	60
GPT	IU/L	25	37	20
ALP	IU/L	2,180	1,570	2,230
GGTP	IU/L	205	88	142
中性脂肪	mg/dl	206	-	80
総コレステロール	mg/dl	232	204	195
リン脂質	mg/dl	397	350	314
尿素窒素	mg/dl	17	8	13
アンモニア	μg/dl	110	—	73
ナトリウム	mEq/L	139	140	138
カリウム	mEq/L	5.4	5.2	5.6
クロール	mEq/L	109	110	108
カルシウム	mg/dl	10.1	9.0	8.6
リン	mg/dl	5.6	6.3	5.3
鉄	μg/dl	119	—	103
総鉄結合能	μg/dl	123	—	151
ビタミンE/総脂質	mg/g	0.9	-	1.0
トロンボテスト				
ビタミン非経口K投与前	(%)	35	24	22
ビタミン非経口K投与後	(%)	95	53	47
手関節X写真				
クル病性変化		有	無	無

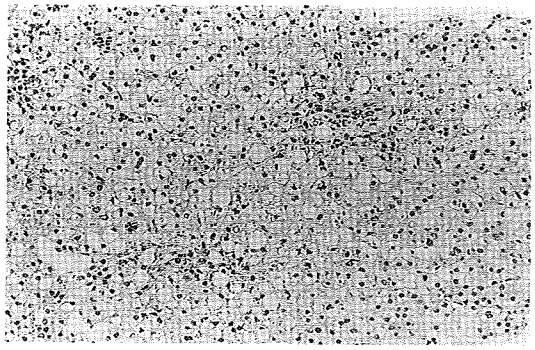

写真1　症例1　瀰漫性の大脂肪滴型脂肪肝（鉄染色：200倍）.

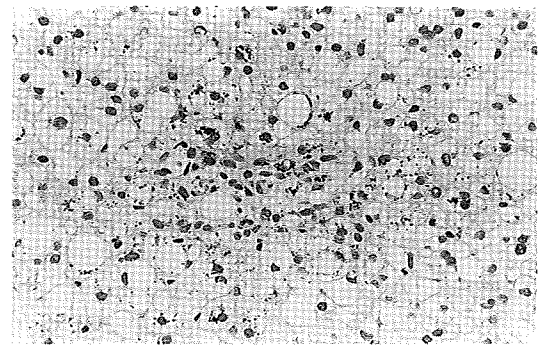

写真2　症例1　瀰漫性のヘモシデリン沈着（鉄染色：400倍）.

肋骨弓下4cm）を認めたほか，白内障が認められた．入院時主要検査所見は表1に示すが，低蛋白血症4.1g/100ml，低アルブミン血症2.7g/100ml，トロンボテスト低活性35%，ガラクトース血症99mg/100ml，高フェリチン血症2,700μg/mlが認められた．ガラクトース代謝関連3酵素活性は，いずれも正常であった．経皮的肝生検による肝組織像は，瀰漫性の大脂肪滴・ヘモシデリンの沈着，軽度の髄外造血巣，軽度の門脈域線維化を認めたが，巨細胞性変性像は認めなかった（写真1～2）．入院後混合栄養をガラクトース除去中鎖脂肪酸含有ミルク（ML-2，森永乳業）に変更後，生化学的検査所見の改善（1週後）・正常化（生後5カ月），体重増加が認められた．

［症例2］生後39日の男子，在胎39週，2,700gにて出

生する．血族結婚歴は認めず，流産歴も認めない．患児は第1子，新生児先天性代謝異常スクリーニングは正常と報告されている．母親には，出生前2週間の鉄剤内服歴がある．生後より人工乳で保育されている．1カ月検診にてヘパプラスチンテスト低値（23%）が指摘され，生後39日，八戸赤十字病院小児科に入院する(体重4,540g)．入院時所見としては，黄疸，灰白色便，濃黄色尿を認めたが，肝・脾腫大は認めなかった．入院時主要一般検査所見は表1に示すが，低蛋白血症3.2g/100ml，低アルブミン血症2.3g/100ml，トロンボテスト低活性24%，高チロシン血症6.2mg/100ml，高メチオニン血症7.0mg/100mlが認められた．尿サクシニルアセトンは検出されなかった．経皮的肝生検による肝組織像は症例1と同様の所見を示したが，門脈域線維化は軽度であった(写真3)．入院後混合栄養をガラクトース除去中鎖脂肪酸含有ミルク（ML-2，森永）に変更後，生化学的検査所見の改善（1週後）・正常化（生後5カ月）が認められた．

[症例3]生後39日の男子，在胎37週，2,700gにて出生する．血族結婚歴は認めず，流産歴も認めない．患児は第1子，新生児先天性代謝異常スクリーニングにて血中ガラクトースの高値（8mg/100ml）が指摘されたが，ボイトラー法は正常であった．母親には，出生前6週間の鉄剤内服歴がある．生後母乳にて保育されていたが，入院前2週間前より混合栄養に変更されている．1カ月検診にて閉塞性黄疸が指摘され，生後39日，秋田大学小児科に入院する(体重4,040g)．入院時所見としては，黄疸，灰白色便，濃黄色尿を認めたが，肝・脾腫大は認めなかった．入院時主要一般検査所見は表1に示すが，低蛋白血症3.9g/100ml，低アルブミン血症2.6g/100ml，トロンボテスト低活性22%，ガラクトース血症25mg/100ml，高チロシン血症3.7mg/100ml，高フェリチン血症1,791ng/mlが認められた．ガラクトース代謝関連3酵素活性はいずれも正常であった．頬粘膜・骨髄生検では，ヘモジデリンの沈着を認めなかった．経皮的肝生検による肝組織像は症例1～2と同様の所見を示したが，門脈域線維化は軽度であった(写真4)．入院後，混合栄養をガラクトース除去ミルク（ラクトレスミルク，明治乳業）による人工乳栄養に変更後，すみやかな生化学的検査所見の改善（1週後）・正常化（生後3カ月）が認められた．1歳時に施行された肝生検は，軽度の門脈域の線維化のほか，ヘモジデリン・脂肪滴の沈着等の異常所見は認めなかった．

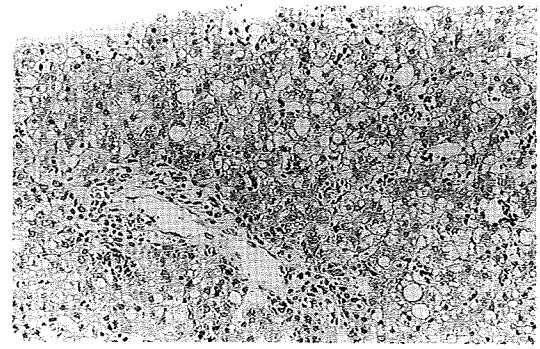

写真3　症例2　溺漫性の大脂肪滴型脂肪肝，溺漫性のヘモシデリン沈着（鉄染色：200倍）．

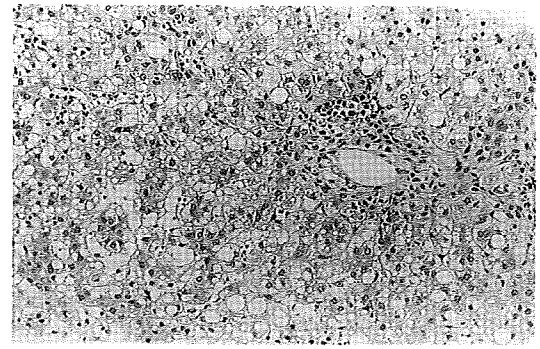

写真4　症例3　溺漫性の大脂肪滴型脂肪肝，溺漫性のヘモシデリン沈着（鉄染色：200倍）．

考　案

新生児肝炎の合併症は多様であるが，病初期に経験する主たるものとしては，脂肪吸収障害に伴う体重増加不良，脂溶性ビタミン欠乏症があげられる．このほか，ガラクトース血症，高チロシン血症，高メチオニン血症，低血糖，低ナトリウム血症，低カルシウム血症，高尿素血症，代謝性アシドーシスなどであるが，長期的には慢性肝炎，肝硬変，成人型肝癌の合併症が問題となる[5]．しかし，低蛋白血症は新生児肝炎の病初期に経験することは極めて稀であり，浮腫・腹水などを伴う肝不全例で経験するのが一般的である．我々の3症例は，著しい低蛋白血症，ビタミンK依存性凝固因子低活性を示したにもかかわらず，非経口的ビタミンKの投与に良好に反応し，正常化すると共に，入院後特殊ミルクへの置換，適切なカロリーと蛋白の供給により，生化学的異常は速やかに改善している．以上の事実は，本症の発症に低栄養が関連していた可能性

が示唆される．

現在まで我々の知る限りにおいて，新生児期に認められる閉塞性黄疸と低栄養に関する報告は認められないが，低栄養と胆汁うっ滞との関連を示す興味深い論文がある．Johnらは栄養上の問題がある低蛋白低栄養の年長乳児4例（4カ月～）を報告しているが，その中の1例（生後7カ月）は，肝腫大・脂肪肝のほか，直接型高ビリルビン血症・高GGTP血症を示している[14]．さらに低蛋白低栄養では脂肪肝のほか，鉄利用が低下しているためにヘモシデリンの沈着が認められる[15]．我々の3症例は離乳開始前であり，低栄養の病因としては母乳あるいは人工乳の投与不足が推測される．症例1，3で新生児期に栄養法が母乳栄養から混合栄養に変更されていること，特に症例1では体重増加不良が認められた事実は母乳不足の存在を示唆するものと考えられるが，症例2～3では，体重増加は一見正常である．新生児・乳児の浮腫は見逃されることが多く，全身性浮腫は「まるまる」とした健康的な印象を与える危険性があり，注意深い観察が要求される[14]．我々の2症例では，全身性浮腫が体重増加不良を隠蔽したと考えられる．しかし，母乳・人工乳投与不足の原因は不明である．患児らの環境は貧困とは無縁であり，教育を十分に受けている両親であり，小児虐待の記録も認めない．

新生児期に閉塞性黄疸の臨床像を示し，肝組織学的に大脂肪滴型脂肪肝を示す疾患としては，多様な代謝性疾患があげられる[8)~13)]．しかし大脂肪滴型脂肪肝と肝ヘモシデローシスの組み合わせは稀な所見であり，主として新生児ヘモクロマトーシス，Zellweger症候群，遺伝性チロシン血症，ガラクトース血症等の代謝性疾患で認められる[11)~13)]．我々の症例では，後3者は臨床像・生化学的所見から否定的であるが，新生児ヘモクロマトーシスとの異同が問題となる．しかし，一般的に新生児ヘモクロマトーシスの発症時期は新生児早期であり，経過は致死的である．さらに，ヘモシデリンの沈着は肝臓に限らず，他臓器に認められる点から，我々の症例とは異なるものと考えられる．

脂肪肝は胆道閉鎖では極めて稀であり，新生児肝炎では一部の症例に認められるに過ぎない[16)17)]．一方ヘモジデリンの沈着は胆道閉鎖・新生児肝炎で共に認められるが，新生児肝炎では一般的に軽微にすぎない[17)~19)]．従って新生児期に認められる閉塞性黄疸では，瀰漫性脂肪肝・肝ヘモジデローシスの組織学的組み合わせは極めて稀な特異な所見と言える．生後1カ月時，肝臓の鉄貯蔵量は極値を示し，ヘモジデリンの沈着も認められるが[20)]，新生児の鉄貯蔵量の固体差は極めて著しいと報告されている[21)]．しかし，脂肪肝の報告は認めない．我々の3症例では，母親の出産直前の鉄剤内服歴が認められる．通常，母親に投与される鉄剤は胎児に影響を与えないとされているが，稀な疾患である新生児ヘモクロマトーシスで推定されているように[12)]，母親を介しての「過剰な鉄の供給に対する胎盤機能は万全でない」可能性を否定できない．過剰な鉄の蓄積は，脂質過酸化，ミトコンドリア傷害による肝細胞壊死，高フェリチン血症，肝内中性脂肪の蓄積，肝線維化を招来し[22)]，胆汁うっ滞が惹起される機序が想定され，その結果としての肝機能傷害により，低栄養がより助長される可能性は否定できない．

我々は，新生児肝炎の臨床像を示し，脂肪肝・肝ヘモジデローシスを伴う特異な組織所見を示す3症例を報告した．その病因としては，鉄負荷，低栄養が推測された．

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Neonatal Cholestasis Associated with Hypoproteinemia —Hepatic Steatosis and Siderosis—

Yusaku Tazawa[1], Daiki Abukawa[2], Yasuro Oyake[3], Fujihiko Nishinomiya[1], Shunichi Maisawa[4], Junichiro Aikawa[2], Toshihiro Ohura[2], Goro Takada[1] and Tasuke Konno[5]

[1]Department of Pediatrics, Akita University School of Medicine
[2]Tohoku University School of Medicine
[3]Hachinohe Red Cross Hospital
[4]Morioka Children Hospital
[5]Department of Pediatric Oncology, Institute for Developing, Aging and Cancer, Tohoku University

Idiopathic neonatal hepatitis (INH) is associated with a variety of complications, but rarely with hypoproteinemia. We experienced three cases with hypoproteinemia, ≦4.1 g/100 ml, in 60 INH cases over the past 10 years. The remaining 57 cases had a range of 4.9 to 7.0 g/100 ml (5.8 ± 0.7 g/100 ml). The three patients, 39 to 53 days of life, had galactosemia (2/2), tyrosinemia (2/3), methioninemia (1/3), a low activity of vitamin K dependent coagulant factors (3/3), hypotransferritinemia (2/2) and hyperferritinemia (2/2). All three patients had diffuse macrovesicular steatosis and siderosis in the liver. No extrahepatic siderosis was found in the patients examined. By substitution mixed or bottle feeding to galactose-free or galactose-free and middle chain triglyceride-rich milk, all three patients quickly responded within one week and recovered. All biochemical abnormalities normalized by five months of life. A follow-up biopsy, examined in one patient at 12 months of life, showed no fat or iron deposits in the liver but residual portal fibrosis. We reported an undescribed subset of INH associated with hepatic steatosis and siderosis. Iron-overload and/or malnutrition may contribute to the pathogenesis of the disease.

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新生児マススクリーニングを契機に発見され，特異なアミノ酸異常を伴った新生児肝炎7例の検討

東北大学医学部小児科[1]，秋田大学医学部小児科[2]，東北大学医学部病態代謝学[3]
大浦　敏博[1]　虻川　大樹[1]　相川純一郎[1]　中川美智子[1]
石澤　志信[1]　田沢　雄作[2]　成澤　邦明[3]　飯沼　一宇[1]

キーワード：新生児肝炎，肝内胆汁うっ滞，新生児マススクリーニング，アミノ酸代謝異常

要　旨

新生児マススクリーニングを契機に発見され，特異なアミノ酸異常を合併した新生児肝炎7例を報告した．血中アミノ酸分析ではメチオニン，チロシンの他トレオニン，シトルリン，リジン，アルギニンが選択的に上昇し，肝の組織学的検討では胆汁うっ滞像と，肝細胞の高度の脂肪変性が特徴であった．脂溶性ビタミン投与，中鎖脂肪酸含有ミルクの使用により2カ月から1年の経過で全例改善し，予後は良好であった．本症は，臨床的には新生児肝炎の経過をとるが，肝組織学的には脂肪変性を呈し，特異な病因を背景にするものと考えられた．

緒　言

新生児マススクリーニングで陽性となった症例においてはスクリーニング対象以外の疾患も存在することが知られている[1]．特に，新生児肝炎例では血中メチオニンやガラクトースの上昇を伴うことがあり，ガスリー検査で陽性となる．今回我々は新生児マススクリーニングで異常を指摘され，精密検査の結果新生児肝炎と診断した症例の中にメチオニンのみならずトレオニン，シトルリン，チロシン，フェニルアラニン，リジン，アルギニン等の増加を認め，肝病理組織学的には胆汁うっ滞と肝細胞の脂肪変性が特徴であった7症例を経験したので報告する．

症　例

症例1：女児，在胎38週自然分娩，2,382gで出生．人工栄養．生後7日目のガスリー検査でフェニルアラニン8mg/dl，メチオニン4mg/dl，13日目の再検査でもメチオニン3mg/dlであった為当科紹介となる．初診時のアミノ酸分析では異常を認めなかったが日齢40の再検査で血中アミノ酸の高値，尿還元糖反応陽性を認め，体重増加不良（14g/日），尿の黄染，黄疸の増強も出現したため日齢61に入院となった．肝は右季肋下に2cm触知した．

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東北大学医学部小児科　　大浦　敏博

症例2：男児，在胎40週自然分娩，2,908gで出生．混合栄養．生後5日目のガスリー検査でフェニルアラニン4mg/dl，13日目の再検査でも8mg/dlと高値であるため16日目に当科受診，フェニルアラニン16.9mg/dlと増加しており日齢22に入院となった．眼球結膜に軽度黄疸，淡黄色便を認めた．肝は1cm触知した．

症例3：女児，在胎43週自然分娩，2,546gで出生．人工栄養．生後4日目より嘔吐を認め3日間補液を施行された．10日目に退院するも体重増加不良（6g/日）のため，産科に日齢16に再入院となった．15日目に行なったガスリー検査でメチオニン2mg/dl，再検でも3mg/dlの為日齢29日に当科転科となった．肝を2cm触知した．黄疸は入院時目立たなかったが，入院後増強した．

症例4：女児，在胎38週自然分娩，2,965gで出生．人工栄養．19日目の保健婦訪問で黄疸を指摘されていた．31日目に行なわれたガスリー検査でメチオニン2mg/dl，ガラクトース6mg/dl，再検でもメチオニン4mg/dl，ガラクトース16mg/dlと高値であったため44日目に当科紹介された．来院時ガラクトース値は正常であったがアミノ酸の高値，肝機能障害を指摘され，日齢58に当科入院となった．肝は辺縁を触知．尿還元糖反応は陽性であった．

症例5：女児，在胎38週吸引分娩，2,958gで出生．人工栄養．第1子はガスリー検査でガラクトース陽性であったが精査の結果否定されている．患児は生後5日目のガスリー検査でフェニルアラニン4mg/dl，メチ

オニン1.2mg/dl，再検査ではいずれも2mg/dl であり19日目に当科紹介となった．来院時アミノ酸の高値，肝機能障害を認めたため日齢39に入院となる．黄疸は生後1週間頃増強したが入院時には軽減していた．便は淡黄色であった．肝は1.5cm 触知した．

症例6：男児，症例5の弟である．在胎40週，胎児仮死により緊急帝王切開，2,514g で出生した．人工栄養．新生児仮死，四肢の硬直を認めた為日齢3に某院小児科入院となる．入院時 GOT 130，GPT 349，ヘパプラスチンテスト10%以下と重度肝機能障害を認め，補液，ビタミンK投与などが行なわれ日齢14に退院した．退院時のガスリーテスト（日齢14）の結果ではガラクトース16mg/dl，メチオニン1.5mg/dl と高値であり，再検査でもメチオニン3mg/dl と異常であった為日齢25に当科受診した．淡黄色便を認め，肝は1cm 触知した．

症例7：男児，在胎40週自然分娩，2,728g で出生．混合栄養．ガスリー検査ではフェニルアラニンが陽性であったが再検では陰性であった．黄疸が持続することより日齢63に胆道閉鎖疑いで某大学病院にて入院，精査の結果新生児肝炎と診断された．転居に伴い日齢110に当科紹介となった．来院時肝機能障害，アミノ酸の高値，体重減少を認めたため精査目的で日齢126に入院となった．眼球結膜に黄染を認めた．肝は2.5cm 触知した．

方法：アミノ酸分析は日立835型アミノ酸自動分析計を用いた．総胆汁酸は酵素比色法（エンザパイル・2，第一化学薬品），ビタミンEは蛍光定量法で測定した．リポプロテインXはリポ蛋白分画試薬（リンタングステン酸-Mg^{++}）を用いて沈殿後 PL-E 試薬（国際試薬）にてリン脂質量を測定して算出した．その他の血液生化学検査は自動分析計を用いた．

検査成績：各症例の血液生化学検査結果を表1に示す．血清総蛋白質，アルブミン，BUN は全例正常であった．いずれの症例においても総胆汁酸の著増が特徴的で高度の胆汁うっ滞を示す所見である．その他，直接ビリルビン，ALP，γ-GTP が上昇しており，GPT は軽度から中等度の増加にとどまっていた．リポプロテイン-X は4例中3例で陽性であった．また，治療前のトロンボテスト，ヘパプラスチンテスト，血中ビタミンE濃度は全例に低値であった．血中アミノ酸分析では全例にメチオニンの上昇に加えトレオニン，シトルリン，チロシン，リジン，アルギニンの選択的上昇が認められ，その他のアミノ酸はいずれも軽度の増減にとどまっていた（図1）．フェニルアラニンは症例2においてのみ上昇していた．なかでもシトルリンの上昇は正常の10～30倍と顕著でありシトルリン血症も否定できないため血中アンモニアを測定したがいずれの症例も正常であり，尿素サイクル異常症は否定された．

B型肝炎ウイルスは全例で陰性であり，サイトメガロウイルス，C型肝炎ウイルス，風疹，単純ヘルペス，トキソプラズマについても検索した限りにおいて有意の抗体価の上昇は認めなかった．

経皮的肝生検：症例2，6を除く5例については経皮的肝生検が行なわれたが，細胆管増生や小葉間胆管内胆汁栓などの所見はみられず，胆道閉鎖症は否定された．組織学的特徴は高度の胆汁うっ滞像と肝細胞の脂肪変性であった（図2）．肝細胞内には多数の小～中脂肪滴が沈着しており，一部に膨化を認めた．肝細胞や毛細胆管内には胆汁栓，胆汁色素を認めるが，炎症

表1 主な検査成績

	正常値	症例1	症例2	症例3	症例4	症例5	症例6	症例7
総ビリルビン(mg/dl)	0.2-1.2	8.2	5.4	8.4	6.2	5.6	3.7	6.8
直接ビリルビン(mg/dl)	0.7	5.0	1.4	5.3	2.6	1.9	2.4	3.9
ALP(IU/l)	22-125	1,064	1,293	435	695	773	524	678
γ-GTP(IU/l)	1-49	177	272	168	121	186	253	100
GOT(IU/l)	4 30	179	94	75	71	62	75	138
GPT(IU/l)	3-28	93	63	22	49	41	43	79
LDH(IU/l)	198-424	922	885	609	712	612	906	708
Ch-E(IU/l)	161 414	232	245	220	197	241	250	158
トロンボテスト(%)	66.9-135.3	46	18.3	49.5	nd	nd	nd	nd
ヘパプラスチンテスト(%)	72.2-120.6	nd	nd	nd	31.4	30.8	43*	63.6*
総胆汁酸(μmol/L)	10	370	367	156	207	257	196	237
リポプロテインX(mg/dl)	-10	178.7	nd	陰性	46.2	43.7	nd	nd
ビタミンE(mg/dl)	0.5-1.5	0.49	0.32	nd	0.46	0.38	nd	0.29

nd：検査せず *：ビタミンK投与後の値

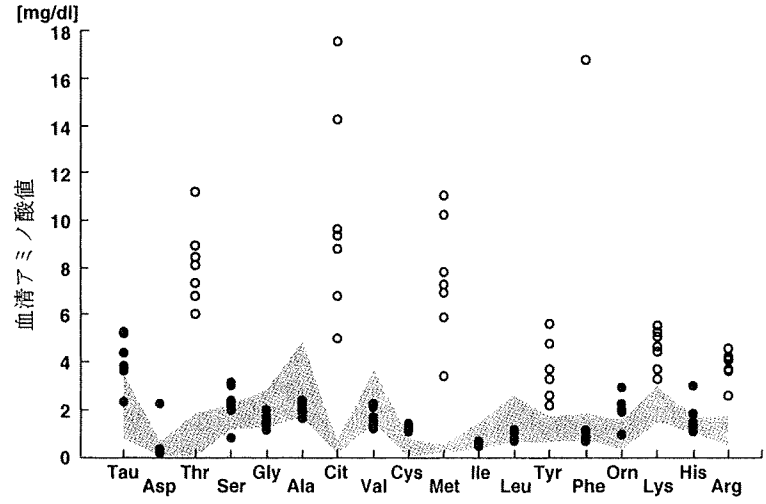

図1　7症例の血中アミノ酸分析の結果.
7症例で高値であったトレオニン，シトルリン，メチオニン，チロシン，リジン，アルギン及び，症例2のフェニルアラニン値を白丸で，他のアミノ酸値を黒丸で示した.
▨：各種アミノ酸の正常範囲

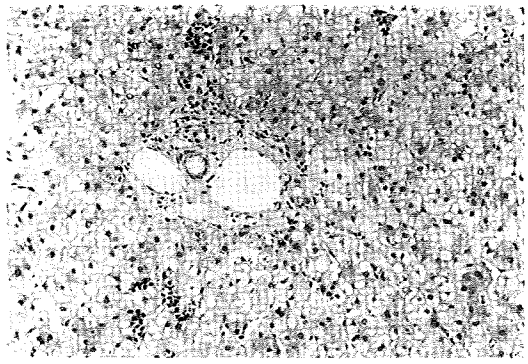

図2　症例3の肝組織像（H-E染色：200倍）
肝細胞内には多数の小～中脂肪滴が沈着している．炎症性細胞浸潤，門脈域の線維化は認めない.

性細胞浸潤，門脈域の線維化は軽度であった．新生児肝炎に特徴的とされる巨細胞変性は見られなかった.

経　過

いずれの症例も高胆汁酸血症を認め，明らかな感染症，解剖学的異常，代謝異常症は否定的であることより新生児肝炎と診断した．全例に脂溶性ビタミン（A, D, E, K）の補給，中鎖脂肪酸含有ミルクの投与を開始した．アミノ酸異常は一過性であり肝機能の改善に先立ち2～6週間で正常化した．肝機能異常は1から4カ月の経過で改善傾向を示したが，トランスアミナーゼの正常化には症例毎に差がみられた．すなわち，正常化した月齢は症例1で17カ月，症例2で7カ月，症例3で12カ月，症例4で12カ月，症例5で7カ月，症例6で5カ月，症例7で8カ月であった．一旦改善しても総胆汁酸，γGTP, GPT値などが再度悪化することがあり，慎重な経過観察が必要であった．今回報告した7例については重大な後遺症を認めた者はなく，1歳時での発育，神経学的予後は良好であった.

考　察

新生児肝炎は生後間もなくから始まる肝内胆汁うっ滞と肝機能障害を主徴とする疾患群で，黄疸，灰白色便（または淡黄色便）および濃黄色尿を伴う．組織学的には巨細胞性肝炎の像をみることが多いが，その病因はまだ確定されるには至っていない[2]．我々の7症例も臨床経過，検査成績より新生児肝炎と診断したが，組織学的には巨細胞性肝炎の像は認めなかった．新生児肝炎症例においては著明な高メチオニン血症や高チロシン血症を呈することがあり先天代謝異常症との鑑別に苦慮することが稀ではない[3)~5)]．また，ガラクトースの上昇を伴うこともあり，新生児マススクリーニング陽性例の中にはこの様な新生児肝炎例が含まれることになる[1]．

7症例の精密検査結果の特徴としては①高胆汁酸血症で示されるように胆汁うっ滞が著明であること，②血中アミノ酸分析ではメチオニン，チロシンに加えト

レオニン，シトルリン，リジン，アルギニンが全例で選択的に増加し，一部にはフェニルアラニンなども上昇すること，③病理組織学的には高度の胆汁うっ滞像と肝細胞の脂肪変性を認めるが巨細胞変性を認めないことが挙げられる．特定の複数のアミノ酸異常を合併した全例に肝細胞の脂肪変性が認められたことは興味深い．これらのアミノ酸異常は肝機能の改善と伴に，特別な治療無しに正常化しており，遺伝的酵素欠損による先天代謝異常症は否定的である．Nishinomiyaら[6]は本邦新生児肝炎例62名の組織学的検索で明らかな脂肪肝を認めたのは10例（16%）であったと報告しているが，アミノ酸値については言及していない．肝脂肪変性はライ症候群に特徴的であり[7]，ミトコンドリアの形態異常を伴うことが多い．しかし，本症例では電顕的検索はなされておらず，ミトコンドリアの異常に関しては不明であり今後の検討課題である．

今回の7例においてはアミノ酸異常は一過性であり，肝機能の改善に先立ち早期に正常化し，その後も肝機能障害だけはしばらく持続している．すなわち肝細胞障害が一次的原因であり，その結果代謝経路を解媒する特定の酵素が障害を受け，前述のアミノ酸異常や脂肪変性が生じるものと推測させる．肝障害の原因は不明であるが症例5と6は姉弟例であることより，本症発症には患者側の遺伝的要因や何らかの感染性病因が関与している可能性は否定できない．

肝内胆汁うっ滞症の治療としては脂溶性ビタミンの投与が重要である[8]．我々の7症例ではビタミンE，K欠乏が全例に，体重増加不良が3例(症例1，3，7)に認められたが，脂溶性ビタミン，中鎖脂肪酸含有ミルクの投与にて，重大な合併症も無く全例肝機能は正常化しており，予後は良好であった．新生児マスクリーニング陽性例のなかには高度のアミノ酸異常を伴い，先天代謝異常との鑑別が問題になる新生児肝炎が含まれることを銘記する必要があると考えられた．

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Disturbances of Amino Acids Metabolism in Infants with Idiopathic Neonatal Hepatitis

Toshihiro Ohura[1], Daiki Abukawa[1], Junichiro Aikawa[1], Michiko Nakagawa[1], Shinobu Ishizawa[1], Yusaku Tazawa[2], Kuniaki Narisawa[3] and Kazuie Iinuma[1]

[1]Department of Pediatrics, Tohoku University School of Medicine
[2]Department of Pediatrics, Akita University School of Medicine
[3]Department of Biochemical Genetics, Tohoku University School of Medicine

Seven infants with idiopathic neonatal hepatitis associated with abnormal aminograms were investigated biochemically and histologically. All patients were detected by newborn metabolic screening. Five infants were positive for homocystinuria, two for phenylketonuria, one for galactosemia. Hypermethioninemia and hypertyrosinemia were previously reported in cases of neonatal hepatitis. In addition to these two amino acids, serum threonine, citrulline, lysine, and arginine levels were elevated. Especially, the citrulline level increased more than ten fold over the upper normal limit (5.0-17.5 mg/dl), but blood-ammonium levels were within the normal range. Serum total bile acids and γGTP were also markedly elevated. Liver histology showed microvesicular fat deposits and severe cholestasis in all patients. Giant cell transformation, which is common in neonatal hepatitis, was not detected. These results were not typical findings of neonatal hepatitis. Abnormal aminograms persisted for a couple of months and improved prior to the disappearance of cholestasis. All biochemical abnormalities normalized by 18 months of life. We speculate that the pathogenesis of the intrahepatic cholestasis associated with abnormal aminograms we reported was different from that of typical neonatal hepatitis.