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Original Article

Plasma citrulline may be a good marker of intestinal functions in intestinal dysfunction

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Abstract ***Background***: Plasma citrulline has been reported to be a good indicator of intestinal functional volume in patients with intestinal dysfunction. We reconfirmed the facts and also investigated the dynamic changes of plasma citrulline in acute-phase patients with intestinal dysfunction.

Methods: We measured plasma citrulline in six patients with intestinal dysfunction who were in the acute and chronic phase for more than 6 months.

Results: Four patients out of six could be withdrawn from total parenteral nutrition, and their plasma citrulline level dynamically changed according to their intestinal states and finally increased up to 15 nmol/mL. Two patients, who could not be withdrawn from parenteral nutrition, showed very low levels of plasma citrulline throughout the treatment course (under 15 nmol/mL).

Conclusion: The cut-off level of plasma citrulline indicating permanent intestinal dysfunction may be 15 nmol/mL in our data. In the acute phase, plasma citrulline changed dynamically according to the intestinal state and may be a good indicator of fluctuating intestinal functions. Thus, although only a few patients were enrolled in this study, plasma citrulline may be a good indicator of stable-state as well as acute-unstable-state intestinal functions.

Key words citrulline, intestinal dysfunction, intestinal function.

In patients with intestinal dysfunction, it is very important to assess whether or not they can be withdrawn from parenteral nutrition, but there has been no easy examination on which to base that judgment. From the early 2000s, the plasma citrulline level has been shown to be a good indicator for the functional intestinal volume in various diseases, such as intestinal failure,[1] short bowels,[2–4] inflammatory bowel disease,[5] villous atrophy diseases,[6] acute mucosal enteropathy due to antineoplastic treatment[7] and bowel transplantation.[8]

We investigated the dynamic changes of plasma citrulline in acute or chronic phases of intestinal dysfunction for more than 6 months. There are still only a few reports available to show the dynamic changes of the citrulline level in adults[9] and pediatric patients[10,11] in the acute phase, and our result suggested that plasma citrulline level is a good indicator of functional intestinal volume in both the acute and stable phases of intestinal dysfunction in pediatric patients.

Methods

We investigated the dynamic changes of plasma citrulline in six pediatric surgical patients (three girls and three boys) who were treated by surgical procedure and/or by parenteral nutrition to improve their general conditions for more than 6 months at Tokyo University Hospital. A retrospective chart review was performed on all six patients, and age, sex, diagnosis, bodyweight, body height, and calorie intake via the parenteral nutrition were recorded.

The Kaup index (body mass index [BMI]) was calculated using the following formula: bodyweight (g)/body height^2 (cm) $\times$ 10. The characteristics of the enrolled patients are listed in Table 1.

Four patients had congenital intestinal diseases, such as intestinal atresia (case 1), meconium peritonitis (case 2), gastroschisis with intestinal perforation and colonic atresia (case 3), and volvulus with malrotation (case 4). Case 5 suffered from refractory enteritis with immunodeficiency. The last patient had undergone resection of almost all of the small bowel at another institution and was brought to our hospital at 2 years of age.

The remnant small bowel length of five of the patients was between 8 and 150 cm, as measured by the surgeon in the operating room and/or on radiograph films. In one patient, the bowel length was not measured.

The blood samples were taken fasting and kept in heparinized tubes; the plasma citrulline level was measured by high-performance liquid chromatography. Routine serum biochemical indexes, such as choline esterase (ChE) and albumin, were all determined automatically by a biochemical analyzer. The normal range of ChE in our institution is 179–354 IU/L and that of albumin is 3.9–4.9 g/dL.

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Table 1 Profile of patients whose plasma citrulline was measured for more than 6 months

Case	Age at entry (months)	Sex	Diagnosis	Bowel length (cm)	Age weaned off parenteral nutrition (months)	Duration of follow up (months)
1	0	F	Congenital jejunal atresia (multiple)	Jejunum: 10 Ileum: 25	5	29
2	0	M	Meconium peritonitis, intestinal atresia	130	1	14
3	0	F	Gastroschisis with intestinal perforation and atresia	85	3	13
4	38	F	Adhesive ileus, malrotation	150	39	26
5	46	M	Refractory enteritis, immunodeficiency	Unknown	(–)	31
6	29	M	Volvulus with malrotation, short bowel syndrome	8	(–)	113

Results

Dynamic changes of plasma citrulline level and the clinical course in four pediatric patients who could be withdrawn from total parenteral nutrition

Case 1 (Fig. 1a)

The female patient was born at 35 weeks and 6 days of gestation with 2420 g bodyweight. She was suspected to suffer from intestinal atresia by fetal ultrasound examination, and it was confirmed by a roentgenogram after birth. She underwent curative surgery at 1 day of age. The superior mesenteric artery was thrombotic, and the residual jejunum was only 10 cm long. The distal intestine was necrotized by ischemia, and only 25 cm of the ileum was alive (Table 1). Intestinal anastomosis was accomplished with peritoneal drainage. The postoperative course was uneventful, and she started enteral feedings with the mother's milk at 18 days after surgery. The plasma citrulline level at 1 month was 6.2 nmol/mL. Intravenous hyperalimentation (70 kcal/kg/day) was started just after surgery but gradually tapered, along with the increase of enteral feeding. Intravenous administration of electrolytes and water was stopped at 5 months of age, and she was discharged at 6 months of age. Serum albumin (3.9 g/dL), ChE and citrulline were within normal limits at the time. The plasma citrulline level increased during the admission period to 15.5 nmol/mL, and it stayed around 15 nmol/mL after discharge. She had normal physical growth, shown as bodyweight gain in Figure 1a.

Case 2 (Fig. 1b)

The male patient was born at 34 weeks of gestation with 2425 g bodyweight. His fetal diagnosis was meconium peritonitis. He underwent drainage at 1 day of life, and curative surgery at 16 days of age. The operative diagnosis was intestinal atresia with fetal intussusception and meconium peritonitis due to rupture of the intestine at the oral side of the intussusception. The residual small intestine was 130 cm (Table 1). Enteral feeding was started at 26 days of age, and he was weaned from parenteral nutrition at 2 months of age. The plasma citrulline level was 10.4 nmol/mL at neonatal age, but parenteral nutrition could be tapered to one-third at 1 month. It stayed around 15–25 nmol/mL after independence from parenteral nutrition, and the patient gained weight and had normal serum albumin (3.9–4.3 g/dL), ChE and Kaup index (BMI).

Case 3 (Fig. 1c)

The female patient was born at 34 weeks and 6 days of gestation with 2222 g bodyweight. She was suspected of having gastroschisis at 16 gestational weeks. The protuberant intestine was ruptured at 31 gestational weeks. She was brought to the operating room immediately after birth and underwent silo placement using the Applied Alexis wound protector (AAWP; Applied Medical Resources Corp, Rancho Santa Margarita, CA, USA) under general anesthesia. The diagnosis at the procedure was gastroschisis with multiple ileum perforations, cecal necrosis and colonic atresia. At 6 days of life, she underwent surgery to close the gastroschisis and construct an ileostomy. But intestinal movement was not well, and she suffered from malnutrition by intestinal dysfunction. Then we performed additional resection of the dilated intestine. The residual intestine was 85 cm at the operation. Thereafter, the residual intestine dilated again, and she received a tube ileostomy for decompression. Her bodyweight gain was good after the serial operations, and she was discharged. The plasma citrulline level was 5.0 nmol/mL, albumin was 2.7 g/dL and ChE was 164 IU/L at 15 days of life and gradually increased to 51.9 nmol/mL, 3.8 g/dL and 327 IU/L, respectively. After the resection of dilated intestine, the citrulline level decreased to 17.8 nmol/mL, but has now re-increased up to 45.3 nmol/mL. In this case, parenteral nutrition was gradually reduced and stopped at 6 months. Her Kaup index (BMI: 15.4) is now in the normal range and bodyweight gain is satisfactory.

Case 4 (Fig. 1d)

The female patient was transferred to our institution for treatment of repetitive ileus and prolonged intestinal dysfunction. She was 3 years old and sustained by total parenteral nutrition. She underwent her first surgery in a local hospital at 1 year and 4 months of age because of duodenal and colonic perforation. Her bodyweight was only 8 kg, and her plasma citrulline level was 13.7 nmol/mL at admission. She had segmental dilatation of the intestine and could not tolerate enteral feeding. We decided to resect the dilated intestine and release the adhesive ileum. The laparotomy was done at 3 years and 3 months of age. The dilated intestine was resected, and the strong adhesions in some intestine were released. The length of the residual intestine was estimated to be 150 cm. Postoperative enteral feeding was restricted because of one episode of bacterial translocation, and parenteral

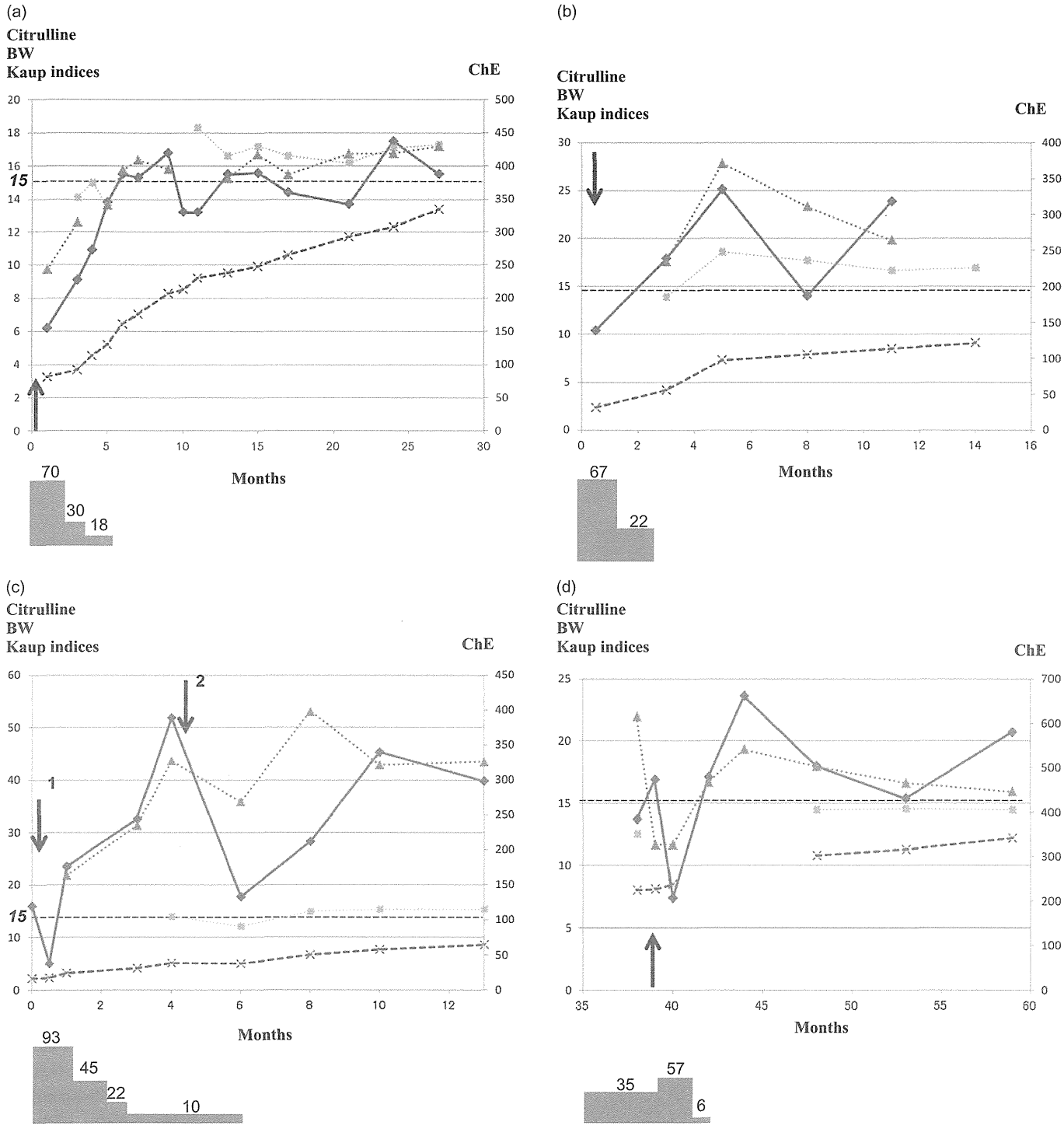

Fig. 1 (a–d) Plasma citrulline levels (Cit), serum cholinesterase (ChE) and calories taken by parenteral nutrition in cases (a) 1, (b) 2, (c) 3 and (d) 4. The dotted horizontal line in each graph represents a Cit of 15 nmol/mL. Black arrows indicate the time of operation. Square boxes under the graph express the parenteral nutrition, and figures written above the boxes indicate the calories administered by parenteral nutrition (kcal/kg/day). (-◆-) Cit (nmol/mL); (···■···) Kaup (body mass index); (-×-) bodyweight (kg); (···▲···) ChE (IU/L).

nutrition (57 kcal/kg/day) was temporarily needed. The plasma citrulline level decreased to 7.4 nmol/mL. Serum albumin (from 4.3 g/dL to 3.1 g/dL) and ChE (from 615 IU/L to 327 IU/L) levels also decreased, and she lost weight. She gradually recovered and became able to tolerate enteral feeding, and at 50 post-operative days she was discharged from our institution. She now lives at home with oral feeding and good weight gain (Kaup index [BMI] was up to 14.5 from 12.5), and ChE level was recovered. The plasma citrulline level was monitored at the outpatient clinic and remained higher than15 nmol/mL.

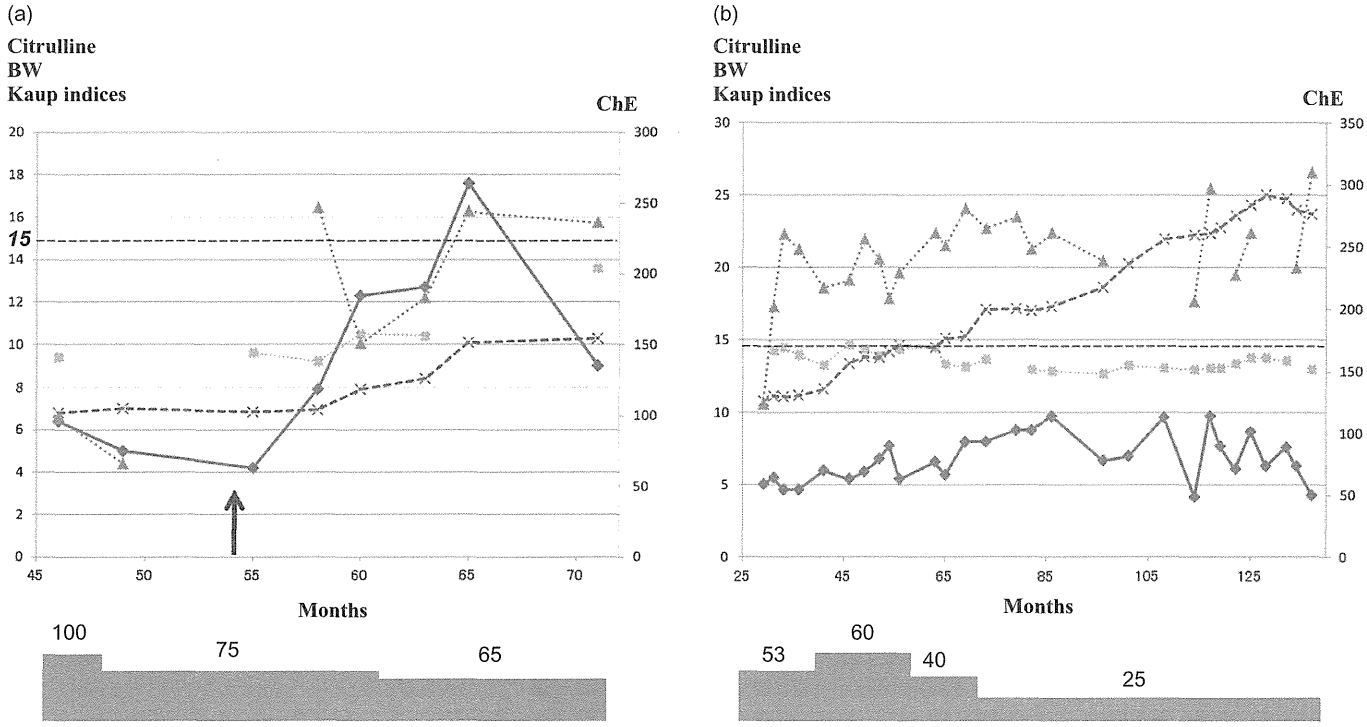

Fig. 2 (a,b) Plasma citrulline levels, serum cholinesterase (ChE) and calories administered by parenteral nutrition in cases (a) 5 and (b) 6, who could not be weaned from i.v. hyperalimentation even with an intensive intestinal rehabilitation program. The dotted horizontal line in each graph represents a plasma citrulline level of 15 nmol/mL. Black arrows indicate the time of operation. Square boxes under the graph express the parenteral nutrition, and figures written above the boxes indicate the calories administered by parenteral nutrition (kcal/kg/day). (-◆-) Cit (nmol/mL); (···■···) Kaup (body mass index); (-×-) bodyweight (kg); (···▲···) ChE (IU/L).

Dynamic changes of plasma citrulline level and clinical course in two patients who could not be withdrawn from parenteral nutrition

Case 5 (Fig. 2a)

The 3-year-old boy was transferred to our institution with severe malnutrition and repetitive enteritis. He had jejunostomy and ileostomy to decompress the intestinal fluid and needed total parenteral nutrition (100 kcal/kg/day) at admission. After admission, intensive care was started, and several examinations revealed that he had some kind of immunodeficiency (we could not identify the type of immunodeficiency and concluded that it was an unknown type). We performed an operation to close the jejunostomy and ileostomy and constructed tube gastrostomy and tube cecostomy to maintain the continuity of the gastrointestinal tract and make the effective decompression possible via those ostomies as well. His enteritis became less severe and the nutritional state improved, but he could not tolerate enteral feeding. Plasma citrulline, albumin, and ChE levels were very low at admission (6.4 nmol/mL, 2.0 g/dL and 98 IU/L, respectively) (Fig. 2a). The Kaup index (BMI) was low (9.2–13.6; Fig. 2a) and bodyweight had been under the normal range even with the support of parenteral nutrition. Intravenous hyperalimentation could not be stopped through the course (65–75 kcal/kg/day).

Case 6 (Fig. 2b)

The 2-year-old boy was transferred to our institution with severe short bowel syndrome. He suffered from midgut volvulus as a neonate due to malrotation of the intestine, and his residual intestine was only 8 cm long. After admission, we started oral feeding but repetitive enteritis and central venous catheter infections occurred, and it was difficult to increase the oral feeding. He is now 11 years old, and the length of his intestine on a radiograph is 32 cm. He could not be withdrawn from the i.v. supplementation of calories and water. Parenteral supplementation was gradually decreased (from 60 kcal/kg/day to 25 kcal/kg/day), but could not be stopped. The plasma citrulline level increased gradually from a level under the detection threshold to 9.8 nmol/mL at 9 years of age, but it was always under 10 nmol/mL. Minimal bodyweight gain was attained but bodyweight had been under the normal range even with the support of parenteral nutrition. He is now scheduled to receive intestinal transplantation but is waiting for a donor.

Discussion

Citrulline is a specific amino acid that is not a component of proteins, and it is mainly synthesized in small intestinal epithelial cells.[12] In the body, citrulline produced by intestinal epithelium is metabolized into arginine in the kidneys. Since the early 2000s

the plasma citrulline level has received a great deal of attention because several reports pointed out that it reflects well the functional intestinal volume in several intestinal disorders, such as short bowel,[2–4] inflammatory bowel disease,[5] intestinal villi atrophy disease,[6] acute mucosal enteropathy due to antineoplastic treatment[7] and intestinal transplantation.[8] Citrulline is contained in watermelon, but oral intake of watermelon does not affect the plasma citrulline level.[3] Plasma citrulline is now being widely tested in clinical areas to assess whether it is a good indicator of the functional intestinal volume.

We reconfirmed the usefulness in our patients. A total of 20 patients who had been treated by parenteral nutrition at one time and now were sustained by enteral feeding had their plasma citrulline level checked (data not shown). Rhoads *et al.*[10] suggested that the citrulline cut-off value for permanent intestinal failure was 20 nmol/mL, but a recent report from the USA demonstrated that it was only 15 nmol/mL.[4] In our data, four patients out of 20 in stable states showed plasma citrulline levels between 15 and 20 nmol/mL (data not shown). In an acute phase, i.v. hyperalimentation could be stopped in four patients, when the plasma citrulline level had reached a final concentration of 15 nmol/mL (Fig. 1). Conversely, two other patients whose plasma citrulline values were less than 15 nmol/mL were difficult to withdraw from the i.v. supplementation (Fig. 2). Our result thus indicates that 15 nmol/mL is the cut-off level for permanent intestinal failure. Crenn *et al.*[3] commented that there may be racial differences in plasma citrulline level and noted that healthy Chinese subjects showed lower values of citrulline than did Caucasians. Nevertheless, our data implied that there is no difference in plasma citrulline levels between Japanese subjects and Caucasians.

Another important point is the relation between citrulline level and intestinal states in our six patients. Intestinal function is mainly defined by the following three factors: (i) total volume of functioning intestinal epithelial cells; (ii) intestinal peristalsis that effectively transports the orally taken nutrients and prevents bacterial overgrowth; and (iii) water and electrolytes absorption mainly seen in the colon. Among these factors, citrulline mainly reflects factor (i). But factor (ii) is also very important for intestinal functions and factors (i) and (ii) are inseparably related. We believe that our total treatment, including cyclic i.v. hyperalimentation (IVH) and probiotics use, was effective in improving factor (i) in cases 1–4. In addition, probiotics were routinely used in severely ill patients to promote intestinal peristalsis and prevent bacterial overgrowth, which improved factor (ii) in cases 1–4. In case 5, however, intestinal function was mainly disturbed by refractory enterocolitis that was caused by systemic immunodeficiency. Such severe enterocolitis could reduce the total volume of functioning intestinal epithelial cells. Thus, we consider that factor (ii) was a critical factor of intestinal failure in case 5 and factor (i) was also affected by severe inflammatory reaction caused by factor (ii) deterioration. In case 6, intestinal length did not increase enough (less than 50 cm) and intestinal dilatation was not seen in spite of our intensive treatment. Therefore factor (i) was the main factor why the patient could not be withdrawn from IVH.

There have been very few reports published showing the dynamic changes of plasma citrulline levels in the acute phase, and in those reports, plasma citrulline was followed for very restricted periods.[9,11] We followed plasma citrulline levels for more than 6 months in six pediatric patients with intestinal failure. Our data demonstrated that the plasma citrulline level changed dynamically according to the intestinal state, and showed that it may be a good indicator of intestinal adaptation after intestinal resection. The plasma citrulline level may also be an indicator of the effectiveness of treatment of intestinal dysfunction and may be used to judge whether or not a patient can be withdrawn from parenteral nutrition. The level of ChE, another nutritional index, was comparatively parallel with the plasma citrulline level; however, the ChE level was within normal range in cases 5 and 6 with parenteral nutrition (Fig. 2), so this indicates that the serum ChE level is not a reasonable indicator to judge whether patients with intestinal dysfunction may be withdrawn from parenteral nutrition. The data presented here are still preliminary, and we need more data on citrulline levels in pediatric surgical children with intestinal failure.

We have shown the diagnostic importance of plasma citrulline measurements, but recently citrulline supplementation has been suggested as an attractive treatment strategy in intestinal failure considering that citrulline is converted to arginine in the kidneys, and arginine plays an important role in compromised hosts.[13] Citrulline supplementation is not yet in clinical use, and more basic research is needed in the future to assess whether citrulline administration will be effective in supporting patients with intestinal failure.[14]

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ORIGINAL ARTICLE

Strategies for catheter-related blood stream infection based on medical course in children receiving parenteral nutrition

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Abstract
Purpose The central venous catheter (CVC) is a useful device for patients requiring parenteral nutrition (PN). However, the risk for catheter-related blood stream infection (CRBSI) is always present. We analyzed the medical course pattern and considered the strategies against febrile events in patients with CVC.
Methods Nine patients receiving PN in our institute from January 2009 to December 2010 were reviewed. Statistical analysis was performed with the Mann–Whitney U test. A p value of <0.05 was considered statistically significant.
Results Eighty-four febrile events were observed. Fifty-six specimens had a positive blood culture, and 52 (93%) specimens were found to be positive in 48 h. The fever dissolved within 48 h in 76 (90%) events after our scheduled treatment. Between the positive and negative blood culture groups, no statistical difference was observed in the count of white blood cell ($p = 0.15$), the proportion of neutrophils ($p = 0.11$) and C-reactive protein ($p = 0.64$). None of the CVCs were removed because of failure to control infection.
Conclusion We recommend the treatment for CRBSI be initiated when patients with CVC develop a high-grade fever, even before exact identification of the cause of infection. The treatment can be corrected after the re-evaluation at 48 h.

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Keywords Catheter-related blood stream infection · Parenteral nutrition · Children · Treatment · Central venous catheter

Background

Parenteral nutrition (PN) is essential for patients with intestinal failure. Intestinal failure is a condition in which the alimentary tract is not able to absorb enough nutrition [1]. For patients who require PN, a central venous catheter (CVC) must be used. As long as the CVC is embedded, there is a risk of catheter-related blood stream infection (CRBSI).

The best therapeutic choice for CRBSI is the immediate removal of the infected catheter [2]. However, the vein that was used for catheter insertion will be occluded once the catheter has been removed. This results in the loss of the available venous access sites.

The loss of the venous access site for CVC insertion can be fatal for patients who depend on PN. Therefore, we have attempted to preserve the CVC during the treatment of CRBSI.

The aim of this study was to search for treatments against CRBSI without removal of the infected CVC by reviewing and analyzing the medical course of CRBSI in patients dependent on PN.

Methods

Nine patients who received PN with CVC over 3 months in our institute from January 2009 to December 2010 were reviewed. The etiologies of intestinal failure of the nine patients enrolled in this study were hypoganglionosis in

five patients, short bowel syndrome in three, and chronic idiopathic intestinal pseudo-obstruction in one. None of the nine patients had other implanted devices such as prosthetic heart valves or vessels. When the patient with CVC developed high-grade fever up to 38°C, we treated them with the same strategy. The strategy involved initiation of treatment for suspected CRBSI immediately if an apparent condition such as pneumonia, influenza or urinary tract infection was not proven. The specimens for blood culture and laboratory tests were collected from the CVC. The CVC was locked and filled with antibiotics (5 mg/ml of amikacin solution) or 70% ethanol. We principally chose antibiotics for filling for the first treatment on admission. But we used ethanol for the events on one patient who insisted on filling with ethanol. A continuous dripped infusion from the peripheral vein and antibiotics were administered for 5 days. The antibiotics were chosen based on the past results of blood culture. When the past results were not referable, cefotiam was used empirically. If the fever dissolved after administration of antibiotics for 5 days, the PN was resumed from the CVC and we ensured no recurrence of developing fever. Unfortunately, when the scheduled treatment failed, the treatment should be corrected, which involved removing the CVC.

The medical data of the patients with suspected CRBSI were collected including the following; (1) the results of blood culture obtained on admission, (2) the period for detection of positive blood culture, (3) the period in which the high-grade fever was observed, (4) the data of laboratory examination and (5) whether the CVC was removed.

The data of each laboratory examination were divided into two groups depending on the result of the blood culture (positive group and negative group). The laboratory examination data were compared between the positive group and negative group. Statistical analysis was performed with the Mann–Whitney U test and a p value <0.05 was considered statistically significant.

Results

During the study period, we experienced 84 events of suspected CRBSI. We locked and filled the CVC with 70% ethanol for the 10 events in one patient who insisted on filling with ethanol. For the 74 other events, we principally chose antibiotics for filling for the first treatment on admission.

In total, 84 blood cultures were performed, as a result, 56 specimens were positive and the other 28 specimens were negative. In the 56 positive specimens, *Escherichia coli* was detected in 19, methicillin-resistant coagulase-negative *Staphylococci* (MRCNS) in 15, *Klebsiella pneumoniae* in 11, methicillin-resistant *Staphylococcus aureus* (MRSA) in 4, *Klebsiella oxytoca* in 3, *Pseudomonas aeruginosa* in 2, *Candida parapsilosis* in 1 and others in 10 (Table 1). In six specimens, multiple species of bacteria were detected.

When the 56 positive blood cultures are viewed from a point of time for positivity, 52 (93%) specimens were found to be positive after a 48-h incubation (Fig. 1). The remaining four specimens that were not found to be positive until after 48 h were considered false positives, resulting from contamination.

When all 84 febrile events were viewed from the point of the febrile period, the fever dissolved within 48 h in 76 (90%) events (Fig. 2). Of the eight events in which the fever persisted over 48 h, three events seemed to be CRBSI from the clinical course. In these three events, *C. parapsilosis*, *K. pneumoniae* and *P. aeruginosa* were detected from blood cultures. We were able to control the infection without removing the CVC in all three events after we had performed CVC locking with 70% ethanol instead of antibiotics. In the remaining five events, the other obvious origin was found from results of the other examination or subsequent clinical course; enterocolitis in two events, primary infection of Epstein–Barr virus in 1, otitis media in 1, and side-effect of vaccination in 1.

In 52 events whose blood culture revealed positive results within 48 h, the fever dissolved within 48 h in 48 events. Of the four events in which the fever persisted over 48 h, one

Table 1 Species of micro-organisms detected from the blood culture

Gram negative bacilli	
Escherichia coli	19
Klebsiella pneumoniae	11
Klebsiella oxytoca	3
Pseudomonas aeruginosa	2
Acinetobacter sp.	1
Pseudomonas putida	1
Enterobacter asburiae	1
Gram positive cocci	
MRCNS	15
MRSA	4
Staphylococcus aureus	1
Streptococcus pneumoniae	1
Micrococcus luteus	1
Enterococcus faecalis	1
Staphylococcus caprae	1
Gram positive bacilli	
Corynebacterium sp.	1
Bacillus subtilis	1
Fungi	
Candida parapsilosis	1
Negative	28

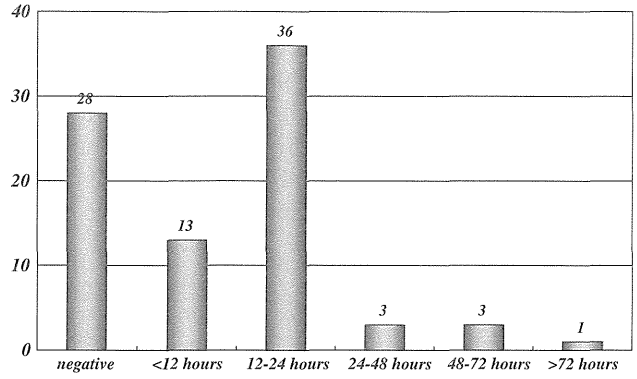

Fig. 1 Time to positivity of blood cultures

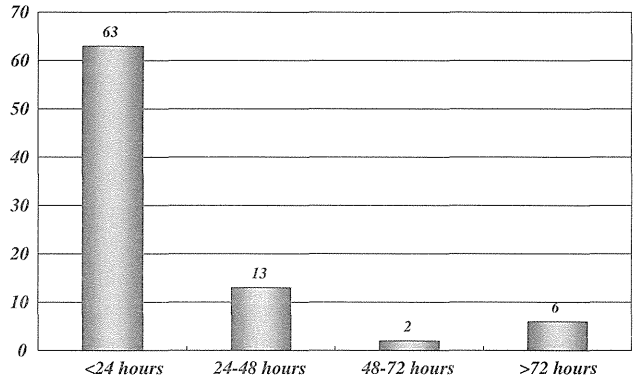

Fig. 2 Time to fever defervescence

event was found to be enterocolitis, whereas three events were recognized as CRBSI. For 28 events with negative culture, it was determined that the febrile event is not caused by CRBSI. Of the 28 events, in 3 events in which the fever persisted over 48 h, another obvious origin was determined. These results are summarized in (Table 2).

Of the 84 laboratory examinations, the 56 exams with positive blood culture were termed the positive group. The other 28 exams were termed the negative group. The count of white blood cell (WBC), the proportion of the neutrophil (%neu) and C-reactive protein (CRP) of each group were investigated as indicators of bacterial infection. The median and interquartile range of each examination is described in (Table 3). No statistical difference was observed in the three examinations (WBC; $p = 0.15$, %neu; $p = 0.11$ and CRP; $p = 0.64$).

During the trial period, no CVC was removed due to failure to control the infection. Replacement of the CVC was performed three times because of breakage or obstruction of the CVC. On all three occasions, we were able to replace the CVC in the same vein.

Discussion

When patients depend on PN, the existence of a venous access site for the CVC must be ensured. Once the catheter has been removed, the vein will be occluded. When the removed CVC reveals a negative culture, the vein may be wastefully occluded. In fact, it is reported that half of the CVCs removed for suspected CRBSI result in a negative culture [3]. When the venous access site for CVC insertion is no longer available, it may be fatal for patients who depend on PN. Therefore, we should strive to treat patients with CRBSI without removing the CVC when they depend on PN.

However, CRBSI is a devastating complication for patients with CVC. The best treatment is to remove the infected CVC [2] when CRBSI occurs. Furthermore, it is reported that the most sensitive and specific technique for diagnosis of CRBSI is the culture of the catheter tip [4]. But removal of CVC may not be the best choice for the patients who depend on PN.

We must consider CRBSI when patients with CVC develop high-grade fever. However, the difficulty of the exact diagnosis of CRBSI lies in the lack of specific

Table 2 Summary of the clinical course of febrile events in our patients

		Blood culture			Total
		Positive		Negative	
		Within 48 h	After 48 h		
Fever resolves within 48 h	Number of events	48	3	25	76
	Diagnosis	CRBSI	Contamination	Viral infection	
Fever persists over 48 h	Number of events	4	1	3	8
	Diagnosis	1 (enterocolitis), 3 (CRBSI)	Infection of EB virus	1 (enterocolitis), 1 (otitis media), 1 (side effect of vaccination)	
Total		52	4	28	84

Table 3 Laboratory test results

	Blood culture		
	Positive	Negative	*p*
WBC	7,305 ± 5,002	8,350 ± 5,565	0.15
%neu	70.3 ± 19.5	65.05 ± 21.4	0.11
CRP	0.41 ± 1.27	0.47 ± 0.92	0.64

Values are expressed in (median ± interquartile range)

symptoms and signs suggestive of CRBSI [5]. It is essential to treat it as soon as possible in a proper way. For prompt initiation of treatment, prompt diagnosis of febrile origin is required.

Blood culture is the required examination to diagnose CRBSI. For diagnosis of CRBSI, quantitative blood cultures and differential time to positivity have a high-degree of accuracy [6]. However, these examinations cannot always be performed. First, it is difficult to collect a suitable sample for blood culture from peripheral vein with suitable procedures because the vessels of the children who depend on PN tend to be exhausted. Furthermore, these procedures cannot be performed in all hospitals. In our hospital, only blood culture obtained through CVC is carried out. Even if the results of this procedure are an overestimation, it is useful as it incorporates necessary conditions of the diagnostic criteria for positive quantitative blood cultures or differential time to positivity. As a result, 52 (93%) specimens were revealed to be positive 48 h after being collected. We regard them as CRBSI, whereas the remaining four specimens were considered false positives with contamination. Shah et al. [7] mentioned similar indication; blood cultures detected 48 h after specimen collection did not involve CRBSI.

Specification of febrile origin is an arduous task. At least, it should be assessed whether the fever was caused by bacterial infection before the antibiotics were administered. In this study, we examined differences between two groups that were divided based on the results of blood culture (positive or negative group). We found no difference between the two groups in terms of the indications for bacterial infection such as WBC, %neu and CRP. This result can be explained by the fact that we collected the blood samples quickly after we had noticed the febrile episode. But it is incongruous to postpone treatment initiation until the laboratory data are observed.

Gram-negative bacilli such as *Escherichia coli* or *Klebsiella* were detected frequently from blood cultures in our study. In many other reports, MRCNS is the species of bacteria detected most frequently [3, 8, 9]. This discrepancy can be explained by the difference of the composition of the underlying disease. In our study, most of the patients suffered from motility disorders of the alimentary tract. On the other hand, in other reports, CVC was applied in most cases for short bowel syndrome. When we analyzed the three patients with short bowel syndrome, a similar trend was observed; we examined the blood culture nine times and revealed positive culture five times. Of the five tests, gram-positive cocci were detected in four tests.

Another important issue is the length of time antibiotics are administered. In the guidelines published by Infectious Disease Society of America, it is recommended that

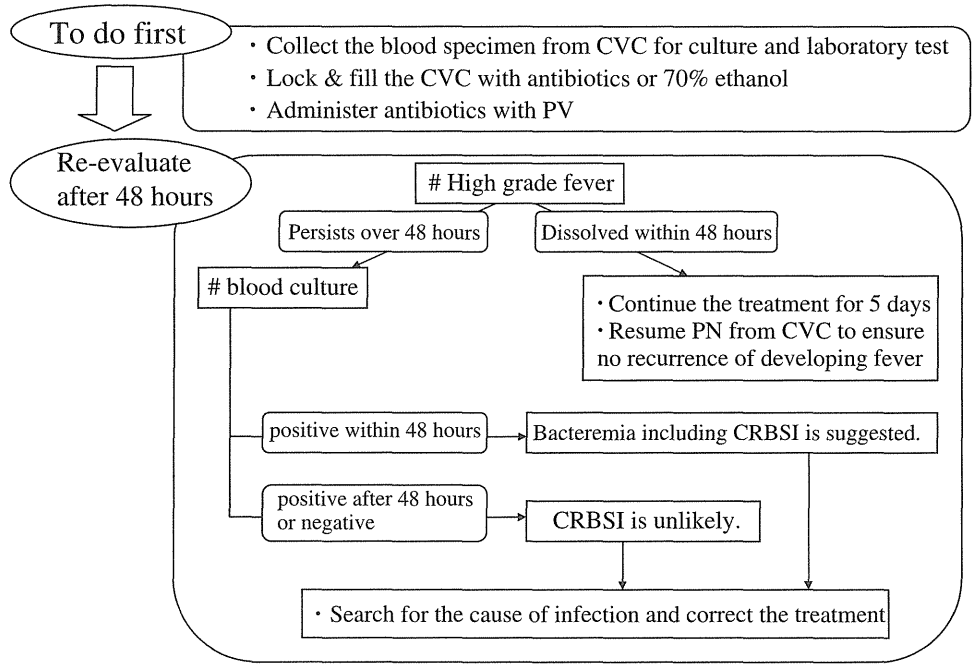

Fig. 3 Our strategy against suspected CRBSI in PN patients

antibiotics should be administered for 10–14 days [10]. In our hospital, infected CVC was locked and filled with antibiotics or 70% ethanol and the antibiotics were administered from the peripheral vein for 5 days. This treatment reduced the patients' fevers within 48 h in 76 (90%) events. When the CVC is locked, the supply of micro-organisms is cut off. Furthermore, the antibiotics or ethanol used in the CVC will destroy the microorganisms.

In conclusion, we recommend that patients with CVC who develop a high-grade fever suggestive of CRBSI begin treatment immediately. Treatment for CRBSI should be started before exact identification of the cause of infection. After 48 h, we can obtain the result of blood culture whether the fever is resolved or not. The treatment can be corrected after the re-evaluation according to the information (Fig. 3). It is noteworthy that using our strategy, no CVC was removed because of failure to control infection.

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ORIGINAL ARTICLE

Effect of an omega-3 lipid emulsion in reducing oxidative stress in a rat model of intestinal ischemia–reperfusion injury

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Abstract
Objectives The usefulness of omega-3 lipid emulsions has been extensively studied. The objectives of the present study were to examine the effect of an omega-3 lipid emulsion in reducing oxidative stress in a rat model of intestinal ischemia–reperfusion injury and the underlying mechanism.
Methods A total of 66 rats were divided into three dietary groups (lipid-free, soybean oil, and fish oil groups). Each animal was administered total parenteral nutrition for 3 days, followed by induction of intestinal ischemia for 100 min. Animals subjected to sham surgery served as the controls. Intestinal tissue and blood were harvested 6 and 12 h after the surgery, then, assessment of the histological damage score, plasma-related parameters, and statistical evaluation were performed.
Results The histological damage score in the intestinal tissues was significantly lower in the fish oil group than in the soybean oil group ($P = 0.0121$). The late-phase urinary level of 8-hydroxy-2-deoxyguanosine was also significantly lower in the fish oil group as compared with that in the other groups ($P = 0.0267$). Furthermore, the plasma level of high-mobility group box 1 protein was also significantly lower in the fish oil group as compared with that in the lipid-free group ($P = 0.0398$).
Conclusion It appeared that intravenous administration of an omega-3 lipid emulsion prior to ischemia–reperfusion injury reduced the oxidative stress and severity of tissue damage. Modification of membrane fatty acids may serve as the mechanism underlying this reduction of tissue damage.

Keywords Ischemia–reperfusion injury · Omega-3 · Oxidative stress · HMGB1

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Introduction

In recent years, the beneficial effects of omega-3 lipid emulsions have been extensively studied in various pathological conditions, including in models of pancreatitis [1], ischemia–reperfusion injury of the small intestine [2, 3], and nonalcoholic steatohepatitis (NASH) [4]. On the other hand, in the clinical setting, acute and chronic administration of general-lipid-strengthened parenteral nutrition has been reported to be associated with elevated risk of liver disorders [5], coronary artery disease, inflammatory bowel disease, and NASH [6].

As an exception to the above-mentioned, omega-3 lipid emulsions have been suggested to reduce the extent of

tissue damage in intestinal ischemia–reperfusion injury by decreasing the oxidative stress. Although this protective effect has been observed in previous studies using rat models of ischemia–reperfusion injury of the small intestine [2, 3], the pathogenesis of the injury and the mechanism underlying the protective effect of omega-3 lipid emulsions against this injury remain poorly understood. The aim of the present study was to evaluate the protective effect of omega-3 lipid emulsions against tissue damage in intestinal ischemia–reperfusion injury, which is commonly seen in ischemic bowel disease, ischemia–reperfusion injury, and small bowel transplantation.

Materials and methods

Experimental animals

Male Crlj: WI rats (250–300 g, 7–8 weeks) obtained from Charles River Laboratories (Yokohama, Japan) were housed in aluminum cages at room temperature (23 ± 3 °C, humidity of 55 ± 3 %) under a 12-h light–dark cycle. All procedures were approved by the Keio University Animal Ethics Committee and Committee on the Animal Experiments of Otsuka Pharmaceutical Factory, Inc.

Experimental design

One week prior to the start of the experiment, the rats were fed a modified AIN-93G diet (Nosan Corporation, Yokohama, Japan), containing a soybean-oil-derived lipid and no fish oil. A central venous catheter (advanced silicon-body plastic tube, 0.5–1.0 mm) was inserted into the internal jugular vein of each rat after the animal had been denied access to food for 12 h, while water was still made available ad libitum. Total parenteral nutrition (TPN) was started on day 0. The rats were divided into three groups (fish oil, soybean oil, and lipid-free groups), and were administered different components of lipids, as shown in Table 1. Omegaven (Fresenius Kabi GmbH, Linz, Austria) was used as the fish-oil-enriched lipid emulsion, and Intralipos Injection 10 % (Otsuka Pharmacy, Naruto, Japan) was used as the soybean-oil-enriched lipid emulsion. TPN was administered for 3 days based upon previous observation by the co-authors of membrane fatty acid changes after 3 days of TPN [7]. Each animal was given a standard caloric supply of 210 kcal/kg/day, corresponding to 30 kcal/kg/day in humans. In the lipid mixture group, 30 % of the total calories were derived from fat. An identical amount of calories was provided by carbohydrates in the lipid-free group. The ratio of amino acids:lipids:glucose of 13:30:57 during TPN was applied according to the recommendation of the European Society for Parenteral and Enteral Nutrition and Metabolism (ESPEN) [8]. On day 3, after a 2-h infusion of extracellular fluid, the rats were subjected to intestinal ischemia–reperfusion, as described below, or sham surgery. After the surgery, the infusion of extracellular fluid, and food and water were withheld. Rats were killed 6 h or 12 h after reperfusion, and intestinal tissue, urine, and blood samples were harvested.

Table 1 Components of infusion solutions

	TPN with lipid-free	TPN with soybean oil	TPN with fish oil
Water volume (mL/kg/day)	260	260	260
Glucose (g/kg/day)	46	30	30
Amino acids (g/kg/day)	7	7	7
Lipids (g/kg/day)	0	7	7
Total calories (kcal/kg/day)	211	211	211

A total of 66 rats were randomly assigned to the following six groups:

1. lipid-free TPN undergoing sham surgery, killed after 6 h ($n = 3$) and killed after 12 h ($n = 5$)
2. soybean oil TPN undergoing sham surgery, killed after 6 h ($n = 3$) and killed after 12 h ($n = 5$)
3. fish oil TPN undergoing sham surgery, killed after 6 h ($n = 3$) and killed after 12 h ($n = 5$)
4. lipid-free TPN undergoing reperfusion surgery, killed after 6 h ($n = 6$) and killed after 12 h ($n = 8$)
5. soybean oil TPN undergoing reperfusion surgery, killed after 6 h ($n = 6$) and killed after 12 h ($n = 8$)
6. fish oil TPN undergoing reperfusion surgery, killed after 6 h ($n = 6$) and killed after 12 h ($n = 8$)

Surgical techniques

The experimental animals were handled as previously reported [9]. General anesthesia was administered by isoflurane inhalation. The superior mesenteric artery was occluded with a clamp, and the small bowels were reperfused after 100 min of ischemia.

Histological assessment of the intestine

5 cm specimens of the small intestine were randomly harvested from a region 10 cm proximal to the terminal ileum and processed for histological examination; after the specimens were fixed in formaldehyde (10 %), they were stained with hematoxylin–eosin. Each intestinal specimen was scored for evaluating the severity of tissue damage using the Park injury scoring system. The scores in this

rubric grade from 0 to 8 (Table 2; [10]). To reduce sampling error, each sample was divided into four parts, and each part was evaluated.

Biomarkers

Urine samples were collected 0–6 h after the reperfusion (early-phase urine samples) and 6–12 h after reperfusion (late-phase urine samples) and preserved in a freezer at −80 °C. Blood samples were collected from the inferior vena cava soon after the animals were killed, and the plasma specimens were stored in a freezer at −80 °C. Plasma levels of oxidative stress markers levels, including 8-hydroxy-2-deoxyguanosine (8-OHdG) and isoprostane, and also the concentrations of prostaglandin E2 (PGE2), and high-mobility group box 1 (HMGB1) protein were measured by enzyme-linked immunosorbent assay (ELISA). (The HMGB1 ELISA kit of Shino-Test Corporation, and oxidative stress marker ELISA kit of Japan Institute for the Control of Aging, NIKKEN SEIL Co., Ltd., were used for this study.)

Statistical analysis

Statistical significance was set at $P = 0.05$. In two-group comparisons, Bonferroni correction was used for adjustment of the significance level ($P = 0.05/2$). Statistical analysis was carried out using *EXSUS* (CAC Corporation), based on the *SAS* (SAS Institute Ltd.). Histopathological scores were statistically compared among the groups using Dunnett's test, F test, student's t test, and the Aspin–Welch test. Urine and blood sample scores are presented as mean ± SD. Dunnett's tests were used for two-group comparisons (i.e., comparison between the lipid-free and soybean oil groups, lipid-free and fish oil groups, and, the soybean oil and fish oil groups).

Table 2 The severity of tissue damage using the Park injury scoring system

Grading	Morphological change
0	Normal mucosa
1	Subepithelial Gruenhagen's space at villus tip
2	Extended subepithelial villus sides
3	Epithelial lifting along villus sides
4	Denuded villi
5	Loss of villus tissue
6	Crypt layer infarction
7	Transmucosal infarction
8	Transmural infarction

Results

Histology of the small intestine

There was no mucosal damage in an intestinal tissue after a sham surgery (Fig. 1). The intestinal tissue specimens from the rats in the soybean oil group exhibited severe mucosal epithelial necrosis and shedding, as well as deep-layer necrosis (Fig. 2d). The tissues from the rats in the fish oil group also showed deep-layer necrosis, but no mucosal epithelial necrosis. The rest of the damage in the fish oil group showed no or only mild intestinal tissue damage (Fig. 2f). The histological damage score was significantly lower in the fish oil group compared with that in the soybean oil group ($P = 0.0121$).

The 8-OHdG levels in the late-phase urine samples were significantly higher in the soybean oil group than in the fish oil group (101.7 ± 38.1 vs. 61.7 ± 21.3 ng/mg creatinine (CRE), respectively, $P = 0.0267$) (Fig. 3).

The PGE2 score, based on the plasma levels of inflammatory eicosanoids, after 6 h of reperfusion tended to be lower in the fish oil group than in the soybean oil group (214 ± 74 vs. 416 ± 258 pg/mL, $P = 0.1737$) (Fig. 4). At 12 h after reperfusion, plasma levels of HMGB1, a mediator of endotoxic shock and sepsis, were significantly lower in the fish oil group (0.763 ± 0.32 ng/ml) than in the lipid-free group (1.4 ± 0.63 ng/ml, $P = 0.0398$) (Fig. 5).

Discussion

Ischemia–reperfusion injury occurs frequently in small bowel transplantation, and often constitutes a major complication of this procedure.

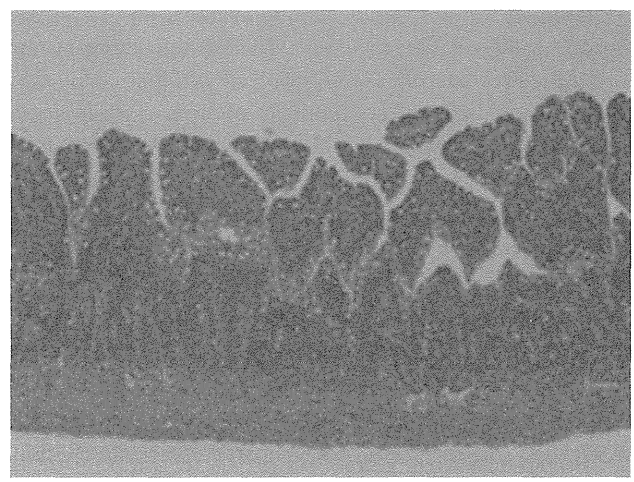

Fig. 1 Histological findings in sham surgery group. No mucosal damage was seen (hematoxylin–eosin staining×100)

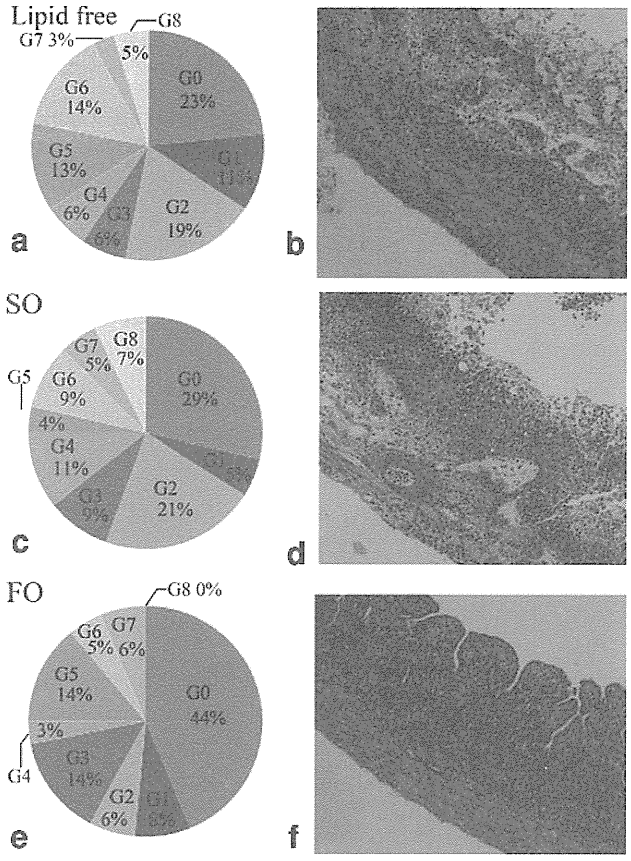

Fig. 2 Representative findings of intestinal tissue specimens after ischemia–reperfusion injury (**b**, **d**, **f**) (hematoxylin–eosin staining×100). Tissue damage scores were also shown (**a**, **c**, **e**). The histological damage score was significantly lower in the fish oil group as compared with that in the soybean oil group ($P = 0.0121$)

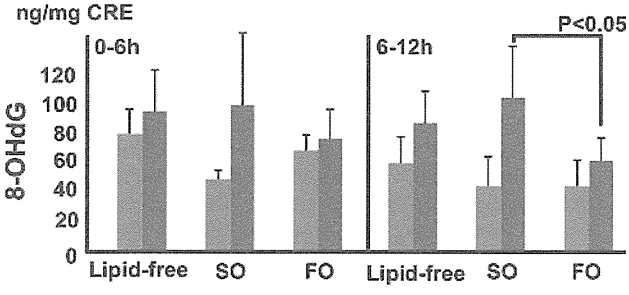

Fig. 3 The 8-OHdG levels were significantly lower in the fish oil group than in the soybean oil group in the late phase (101.7 ± 38.1 vs. 61.7 ± 21.3 ng/mg CRE, respectively, $P = 0.0267$)

In the literature, the mechanism underlying intestinal ischemia–reperfusion injury is typically described as a cascade (Fig. 6). Ischemia–reperfusion injury causes an increase in intracellular calcium, which triggers phospholipase activation and up-regulation of inflammatory eicosanoids, such as the '4' series of leukotrienes (LT), '2' series of thromboxane (TX), and prostaglandin (PG). With

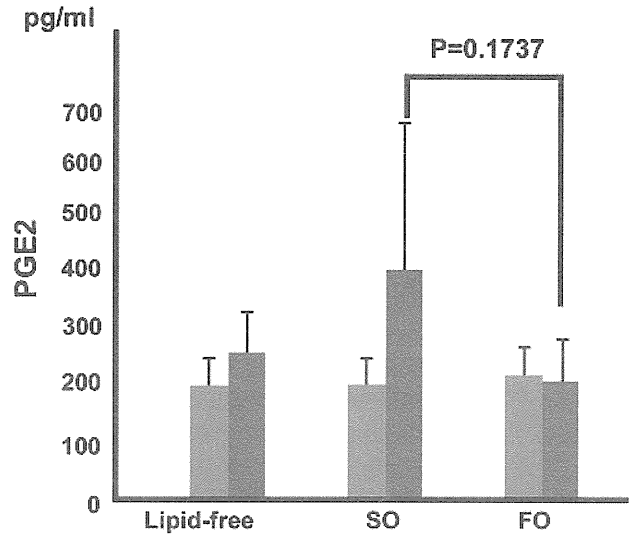

Fig. 4 The PGE2 score at 6 h tended to be lower in the fish oil group than in the soybean oil group (214 ± 74 vs. 416 ± 258 pg/mL, $P = 0.1737$)

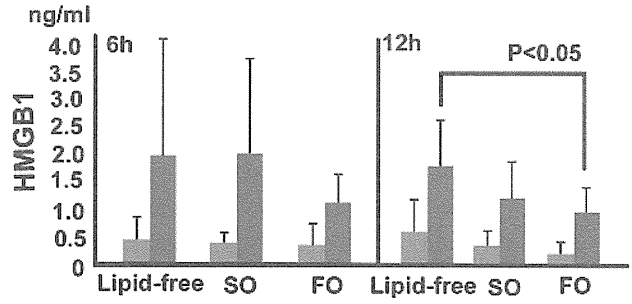

Fig. 5 The plasma levels of HMGB1 were significantly lower in the fish oil group than in the lipid-free group (0.763 ± 0.32 vs. 1.4 ± 0.63 ng/ml, $P = 0.0398$)

the formation of reactive oxygen species (ROS), including superoxide and hydroxyl radicals, nuclear oxidation occurs, resulting in the formation of 8-OHdG as a metabolite. Urinary 8-OHdG formed by nuclear peroxidase appears in the urine in the late-phase of ischemia–reperfusion injury, particularly after 24 h [11, 12]. The production of ROS is associated with induction of cell membrane damage. In addition, neutrophils and macrophage are also activated, increasing the production of inflammatory cytokines. This process also leads to the production of HMGB1, which has been regarded as a mediator of late-phase inflammatory signaling in ischemic injury of organs such as the lung and liver [13, 14]. A characteristic finding of small-intestinal injury was that the plasma HMGB1 concentrations increased more rapidly as compared with that following injury to other organs [15, 16]. Lower plasma levels of HMGB1 were found in the fish oil group as early as at 6 h after the ischemia–reperfusion injury in the present study.

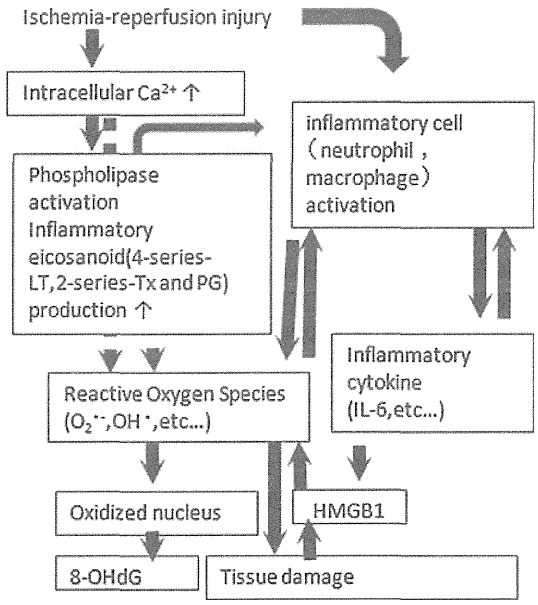

Fig. 6 The mechanism underlying intestinal ischemia–reperfusion injury is typically described as a cascade

Fatty acids derived from fish oil, such as eicosapentaenoic acid (EPA), result in the formation of the '5' series of PGs, TXs, and LTs. These substances are thought to down-regulate the inflammatory response [7]. In contrast, fatty acids derived from soybean oil, such as arachidonic acid (AA), result in the formation of the '2' series of PGs and TXs and the '4' series of LTs, which promote inflammatory responses [7]. Omega-3 lipid emulsions have been reported to down-regulate the production of inflammatory cytokines, such as IL-6 and TNFα [17]; however, only a limited amount of evidence has been accumulated. The present study was aimed at investigating the effect of fish-oil-derived fatty acids against ischemia–reperfusion injury of the intestine, as compared to other lipid components. The severity of the injury was assessed by evaluation of the changes in the plasma levels of inflammatory markers and oxidative stress markers, and the histopathologic tissue damage scores.

In the current study, fish oil administration significantly reduced the severity of histological damage in the fish oil group as compared with that in the soybean oil group. Reduction in the plasma levels of oxidative stress markers was observed, along with a decrease of the plasma HMGB1 levels. Therefore, the present observations indicate that the severity of tissue damage was reduced through down-regulation of oxidative stress and inflammatory responses. In addition, lower plasma levels of inflammatory eicosanoids observed in the present study also suggest that attenuation of the change in the omega-3/omega-6 ratio in the membranous lipid may play a major role in reducing the tissue damage. In the data, a few outliers made fairly large SD, especially in HMGB1 and PGE2. In addition to the delicate surgical animal model, dynamic changes of these parameters in vivo could result in these variabilities.

Prior administration of the omega-3 lipid emulsion reduced the plasma/urinary levels of inflammatory markers both in the early and late phases of ischemia–reperfusion injury. Consistent with the results of the current study, Byrne et al. [3] reported suppressed neutrophil adherence, which reduced the severity of ischemia–reperfusion injury, and also that omega-3 lipids mimic the early events in the injury. Furthermore, Sukhotnik et al. [2] reported decreases in the severity of intestinal mucosal injury and enterocyte apoptosis following intestinal ischemia–reperfusion injury in the rat.

These observations, including our own, suggest that the efficacy of fish oil may be attributable not only to a single step action in the late phase, but also to several steps in the inflammatory cascade. Therefore, administration of omega-3 lipids prior to intestinal ischemia may exert a significant beneficial effect against intestinal tissue injury.

Our current results indicate the clinical efficacy of omega-3 lipids in reducing intestinal ischemia–reperfusion injury commonly seen after intestinal transplantation. Future studies on omega-3 lipids are warranted for clarifying the mechanism of anti-inflammatory effect more precisely, and subsequently to establish the clinical efficacy of the lipids in a variety of critical conditions.

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Intracranial Hemorrhage Associated With Vitamin K–deficiency Bleeding in Patients With Biliary Atresia: Focus on Long-term Outcomes

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ABSTRACT

Background and Aim: The prophylactic oral administration of vitamin K to newborns has markedly reduced the incidence of vitamin K deficiency (VKD); however, intracranial hemorrhage (ICH) is still one of the complications found in biliary atresia (BA) patients and is associated with VKD bleeding. Therefore, we aimed to investigate the incidence and long-term outcome of ICH in patients with BA who previously received prophylactic vitamin K during the neonatal period.

Methods: Eighty-eight consecutive infants with BA were treated and followed up at Kyushu University Hospital from 1979 to 2009. The clinical records and imaging study results were retrospectively reviewed in the infants with BA who presented with ICH.

Results: ICH occurred in 7.95% of patients with BA. The onset of ICH occurred at 47 to 76 days after birth, before the patients underwent surgery for BA (9–37 days after the onset of ICH). Coagulopathy was found upon admission in all of the cases with available data and improved after intravenous administration of vitamin K. A craniotomy was required in 2 cases before the surgery for BA. During the 22 to 278 months of follow-up, some neurologic sequelae persisted in 5 of 7 cases. Follow-up head computed tomography scans showed a low-density area in the left hemisphere in 5 cases.

Conclusions: Although vitamin K prophylaxis had been given during the neonatal period, ICH-associated VKD bleeding was still found in 7.95% of patients with BA. Persistent neurologic sequelae were found in 5 of 7 cases, with low-density area in the left hemisphere.

Key Words: biliary atresia, intracranial hemorrhage, vitamin K–deficiency bleeding

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Biliary atresia (BA) is a rare disease with an occurrence rate of 1 in 8000 to 18,000 live births (1). The clinical symptoms of BA characteristically include jaundice and acholic stools at 1 or 2 months after birth. Although extremely rare, the presentation of a bleeding disorder as the first symptom of BA has also been reported (2). Intracranial hemorrhage (ICH) is one of the complications found in patients with BA caused by late-type vitamin K–deficiency bleeding (VKDB), which occurs most commonly at the age of 1 to 2 months (3,4). This complication causes not only mortality but also significant long-term morbidity in the survival BA before and even after liver transplantation (2,3,5,6).

Vitamin K is required for the synthesis of coagulation factors II, VII, IX, and X by the liver (7); however, the vitamin K level in the newborns is usually low because of the insufficient vitamin K stores of the newborn and low placental transfer of vitamin K (8,9). In Japan, prophylactic oral administration of vitamin K to newborns at birth, on the sixth day after birth, and 1 month after birth beginning in 1981, has markedly reduced the incidence of idiopathic vitamin K deficiency (VKD) (5). Unfortunately, this prophylaxis has no effect on secondary VKD caused by malabsorption of vitamin K caused by cholestatic disorders such as BA (6).

Although BA had been reported to be one of the major causes of secondary VKD, there are only a few reports on patients with BA presenting with ICH, especially regarding their long-term outcome. In the present study, we describe 7 patients with BA presenting with ICH. We investigated the incidence of ICH in patients with BA who previously received prophylactic oral administration of vitamin K in the neonatal period. Moreover, we also describe the management of BA-associated VKDB complicated by ICH and also focus on the long-term outcome of these patients.

METHODS

Between 1979 and 2009, 88 consecutive infants with BA underwent surgery for BA and were followed up in the Department of Pediatric Surgery of Kyushu University Hospital. Infants who presented with ICH were enrolled in the present study. Their clinical records and imaging studies were retrospectively reviewed. ICH caused by VKDB was diagnosed based on clinical and neurologic signs and symptoms, hematologic examination, and findings on computed tomography (CT) scans of the head. A confirmed case of VKDB should fulfill the diagnostic criteria of at least 2 of the following: hepaplastin level <10% and/or thrombo test <10%; prothrombin time (PT) percentage <10%; activated partial thromboplastin time (APTT) >120 seconds; protein induced by vitamin K absence (PIVKA)-II level exceeding normal controls; improvement of bleeding tendency and PT after 24 hours of vitamin K administration, and by a normal or raised platelet count (7,10).

BA was diagnosed based on clinical presentation including jaundice and acholic stools, hematologic examination, and ultrasonographic findings, and was confirmed by surgical cholangiography. BA types are classified using anatomical classification by Morio Kasai (11).

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Long-term outcomes focusing on neurologic sequelae, such as developmental delay, mental retardation, epilepsy, and hemiparesis, were observed until the end of the follow-up period. Developmental delay was defined as a subset of developmental disabilities in 1 or more developmental domains: gross/fine motor, speech/language, cognition, social/personal, and activities of daily living, on age-appropriate, standardized norm-reference testing. Mental retardation was defined as a condition characterized by intellectual ability that is significantly below average (specifically intelligence quotient [IQ] $\leq$70) combined with deficits in adaptive abilities. Developmental delay assessment or developmental quotient if available was done by a pediatrician in our hospital, whereas IQ and mental retardation assessment was made by a psychologist/psychiatrist. The development was evaluated in 3 aspects: motor, social, and speech development.

RESULTS

Of the 88 consecutive infants with BA, 7 infants (all girls) presented with ICH (7.95% of patients with BA). The age of onset of ICH ranged from 47 to 76 days (mean 62.0 days), with vomiting, consciousness disorders, seizures, dyspnea, anisocoria, and conjugate deviation as the major symptoms at presentation (Table 1).

All of the patients were born full-term and received oral prophylactic vitamin K during the neonatal period. Neither perinatal complications nor any history of head trauma were found for these infants. During the first 6 months of life, 4 patients received exclusive breast-feeding (BF), 1 patient formula milk (FM) containing vitamin K supplementation, and 1 patient mixed BF and FM (Table 1). Although all of the patients were screened at 1 month of age by an obstetrician, VKDB still occurred before the diagnosis of BA was established. After stabilization of their ICH, 5 patients underwent the Kasai operation and 2 patients underwent hepaticojejunostomy for definitive diagnosis of BA. The timing of the Kasai procedure was 9 to 37 days (mean 22.3) after the onset of ICH (Table 1). The types of BA found during surgery were III-b1-*v* in 3 patients, III-b2-*o* in 1 patient, III-a1-*v* in 1 patient, and I-cyst in 2 patients.

CT scans of the head demonstrated that intraparenchymal hemorrhage was found in 4 cases, subarachnoid hemorrhage in 2 cases, and subdural hemorrhage (SDH) in 3 cases. A midline shift (MS) caused by massive hemorrhage was found in 5 cases; however, only 2 of these patients underwent urgent surgical evacuation of an intracranial hematoma before laparatomy because of anisocoria. Consistent with CT evaluation upon admission, magnetic resonance imaging/magnetic resonance angiography (MRI/MRA) during hospitalization (first–fifth weeks after admission) showed a more detailed description of ICH and its complication to the brain such as the development of encephalomalacia in middle cerebral artery territory in 5 patients, cerebral atrophy in 2 patients, cerebral ventricle enlargement in 2 patients, and atrophic of corpus callosum in 1 patient. During follow-up, 2 patients underwent living-related donor liver transplantation (LDLT) and 3 patients died at the ages of 33, 36, and 278 months of liver failure because liver transplantation could not be performed (Table 2).

Upon admission, routine laboratory examination showed platelet levels within the normal range (range 20.8–61.1 $\times$ 10^4 cells/μL), whereas severe anemia was found in 2 patients (range 4.1–4.6 g/dL). Elevated direct bilirubin was found in all of the patients, and elevated alanine aminotransferase and aspartate aminotransferase were found in 6 of 7 cases (Table 3).

Significant prolonged PT and APTT were found in coagulation examination during admission in the patients for whom data were available, with a range of 36.2 to >200 seconds and 67.3 to >200 seconds, respectively. Right after the diagnosis of VKDB was made, intravenous (IV) vitamin K was administered to all 7 patients and fresh frozen plasma (FFP) was given to 5 patients. Follow-up examinations showed improvement in PT and APTT levels from 11.7 to 18.5 seconds and 26.4 to 40.3 seconds, respectively. An elevated PIVKA-II level was also noted in 4 cases (Table 3).

At follow-up examination at 22 to 24 months, developmental delay in all aspects of evaluation (motor, social, speech) was seen in 4 patients, cognitive impairment in 1 patient, and normal development in 2 patients. In 1 patient who had normal developmental evaluation, a mild hemiplegia of the right extremities was observed with upper extremity stronger than the lower 1; however, overall evaluation was normal development. At this point of age, an electroencephalography (EEG) examination was also performed in 4 patients, 3 of whom showed abnormal EEG. One of 3 patients who did not have an EEG evaluation at this time point (case 3) showed clinical epilepsy during follow-up. At this time point, evaluation of the liver function showed elevation of aspartate aminotransferase, alanine aminotransferase, and direct bilirubin in 5 patients (Table 4).

During the 22 to 278 months (mean 90.3) of follow-up, some neurologic sequelae persisted in 5 of the 7 cases. The types of neurologic sequelae found were mental retardation in 2 patients, epilepsy in 1 patient, hemiparesis in 2 patients, and developmental delay in 1 patient who showed bilateral cerebral atrophy. In 2 patients, no neurologic deficits were observed until the end of the follow-up. Follow-up CT scans of the head demonstrated significant ischemic changes shown as a low-density area (LDA) consistent with encephalomalacia in the left hemisphere were found in 5 patients (Table 5). In the present study, a comparison of head CT scans at admission and latest CT at follow-up showed that in case 6, the head CT showed SDH and MS at admission. During follow-up, head CT did not show any new hemorrhage; however,

TABLE 1. Patient characteristics and symptoms at admission

Case	Sex	Infant milk until 6 mo	Onset of ICH, d	Symptoms	Surgery for BA, d	Type of BA (10)
1	F	BF	50	Vomiting	59	III-b1-*v*
2	F	BF + FM	54	Consciousness disorder, seizures	70	III-b1-*v*
3	F	BF	66	Dyspnea, anisocoria	88	III-b1-*v*
4	F	BF	76	Dyspnea, conjugate deviation	110	III-b2-*o*
5	F	BF	47	Vomiting, seizures	84	III-a1-*v*
6	F	FM	69	Consciousness disorder, anisocoria, conjugate deviation, seizures	93	I-cyst
7	F	No data	72	Vomiting	86	I-cyst

BF = breast-feeding; FM = formula milk containing vitamin K supplementation.