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Original Article

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Parenteral Nutrition-Associated Cholestasis in Very Low Birth Weight Infants: A Single Center Experience

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Purpose: Parenteral nutrition (PN)-associated cholestasis (PNAC) is one of the most common complications in very low birth weight infants (VLBWIs). The aim of this study is to evaluate the risk factors of PNAC in VBLWIs.
Methods: We retrospectively reviewed the medical records of 322 VLBWIs admitted to the neonatal intensive care unit of our hospital from July 1, 2009 to December 31, 2013. We excluded 72 dead infants; 6 infants were transferred to another hospital, and 57 infants were transferred to our hospital at 2 weeks after birth. The infants were divided into the cholestasis and the non-cholestasis groups. PNAC was defined as a direct bilirubin level of ≥2.0 mg/dL in infants administered with PN for ≥2 weeks.
Results: A total of 187 VLBWI were enrolled in this study; of these, 46 infants developed PNAC. Multivariate logistic regression analysis showed that the risk factors of PNAC in VLBWI were longer duration of antimicrobial use (odds ratio [OR] 4.49, 95% confidence interval [95% CI] 4.42-4.58), longer duration of PN (OR 2.68, 95% CI 2.41-3.00), long-term lack of enteral nutrition (OR 2.89, 95% CI 2.43-3.37), occurrence of necrotizing enterocolitis (OR 2.40, 95% CI 2.16-2.83), and gastrointestinal operation (OR 2.19, 95% CI 2.03-2.58).
Conclusion: The results of this study suggest that shorter PN, aggressive enteral nutrition, and appropriate antimicrobial use are important strategies in preventing PNAC.

Key Words: Parenteral nutrition, Cholestasis, Very low birth weight infant

INTRODUCTION

Providing parenteral nutrition (PN) in the neonatal intensive care unit (NICU) is an important means of supplying nutrients to promote the growth of premature infants with a birth weight ≤1,500 g. Due to the risk of necrotizing enterocolitis (NEC) and feeding intolerance caused by the impaired gastrointestinal (GI) motility of very low birth weight infants (VLBWIs), many neonatologists hesitate about accelerating the rate of feeding of very-low-birth weight infants. For this reason, VLBWIs are given

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nutrients through PN. However, administrating PN for a long time may induce complications, the most common of which is PN-associated cholestasis (PNAC). The risk factors of PNAC include low birth weight, prematurity, PN duration, absence of enteral nutrition (EN), quality or quantity of amino acid intake, cumulative amount of lipid infusion, toxicity of trace elements, GI operation, shock, and sepsis [1,2].

To help in the care of VLBWIs in the future, this study aims to describe the risk factors of PNAC in VLBWIs at our NICU.

MATERIALS AND METHODS

Patients and criteria

All VLBWIs who received PN for ≥14 days and were admitted to our children's hospital's NICU between July 1, 2009 and December 31, 2013 were included in the study. This was a retrospective study, with follow-up until discharge. We excluded dead infants, infants with major congenital anomaly, infants who were transferred to another hospital, and those who were transferred to our hospital at 2 weeks after birth. The infants were divided into the cholestasis and the non-cholestasis groups. PNAC was defined as an elevation of conjugated bilirubin to ≥2.0 mg/dL in at least one laboratory test during hospitalization. This study was accredited by the Institutional Review Board of the Pusan National University Yangsan Hospital (study no. 05-2014-010).

Characteristics of PN and enteral nutrition

PN was started within the first day of life for all VLBWIs. Dextrose infusion was started at 6 mg/kg/min and increased to a maximum of 10-12 mg/kg/min and adjusted according to the blood sugar level, calorie intake, and clinical conditions. Administration of amino acids (10% Primene; Baxter, Newbury, UK) was started at birth and increased from 2 g/kg/day to a maximum of 4 g/kg/day at each stage. Fish oil-based lipids (20% SMOFLipid; Fresenius Kabi AG, Graz, Austria) were started at 0.5 g/kg/day and increased daily by 0.5 g/kg/day to a maximum of 3 g/kg/day. Lipids were stopped when EN reached calorie 100 mL/kg/day. Trace elements (Furtman; JW Life Science, Dangjin, Korea) were mixed according to the body weight at each PN administration after the first day of life.

When the infants were clinically stable, EN was started through a nasogastric or orogastric tube as soon as possible. Feeding of VLBWIs was started with breast milk or premature infant formula. EN was started at 10-30 mL/kg/day. The feeding volume was increased by 10-30 mL/kg/day according to gestational age (GA); the goal feeding volume was 150 mL/kg/day. Ursodeoxycholic acid was administered at 15 mg/kg/day for the cholestasis group until the conjugated bilirubin level recovered to <2.0 mg/dL.

Risk factors and definitions

Small for GA was defined as a birth body weight (BBW) below the 10th percentile for GA on a fetal growth chart. The diagnosis of respiratory distress syndrome was based on chest radiograph results together with clinical symptoms. In our NICU, infants with hemodynamically significant patent ductus arteriosus (PDA) were treated with indomethacin or ibuprofen before ligation. The infants received fluconazole prophylaxis of 3 mg/kg/dose through intravenous infusion twice a week for 6 weeks. Antimicrobials included antibiotics and antifungal agents. Congenital hypothyroidism was confirmed with blood thyroid function tests; infants with hypothyroidism were administered with levothyroxine. NEC was defined as Bell's stage ≥2. The diagnosis of sepsis was made based on positive blood cultures in association with the symptoms of systemic inflammatory response syndrome, such as fever, hypothermia, hypotension, and unstable vital signs. Bronchopulmonary dysplasia (BPD) was defined as oxygen supplementation requirement at 36 weeks postmenstrual age. Intraventricular hemorrhage was defined as stage ≥3. Retinopathy of prematurity (ROP) was defined as stage ≥2 plus. Neurosensory hearing loss was confirmed with an auditory brainstem response test. Skeletal fracture was confirmed with radiographic examination. Near-full EN was defined as an enteral tolerance of 120 mL/kg/day. Full EN

was defined as enteral tolerance of at least 150 mL/kg/day.

Infectious causes of cholestasis (hepatitis A, hepatitis B, hepatitis C, toxoplasmosis, rubella, herpes simplex virus, and cytomegalovirus) were excluded in this study. We investigated a-1 antitrypsin, alpha fetoprotein, gamma glutamyl transpeptidase, and the possibility of surgical causes of conjugated hyperbilirubinemia (such as biliary atresia, choledochal cyst on abdominal ultrasonography, and hepatobiliary scintigraphy); there were no abnormalities in our VLBWIs.

Statistical analyses

The Statistical Package for Social Sciences version 21.0 (IBM Co., Armonk, NY, USA) software was used for data analysis. Categorical variables were compared between the two groups by using Fisher's exact test and Pearson's chi-square test. Continuous variables were compared between the two groups by using Student's *t*-test. Due to the differences in GA and BBW between the two groups, these two factors were corrected with an analysis of covariance (ANCOVA). Logistic regression analysis was performed to eliminate interactions between predicted factors of PNAC; the role of specific factors that may affect PNAC was defined. A probability *p*-value of <0.05 was used to establish statistical significance.

RESULTS

A total of 322 VLBWIs were admitted to our hospital during the study period. Of these, 135 infants were excluded: 72 infants who died before discharge, 6 infants who were transferred to another hospital, and 57 infants who were transferred to our hospital at 2 weeks after birth. Thus, a total of 187 infants were enrolled in this study and 46 of these had a diagnosis of cholestasis.

Perinatal characteristics and neonatal demographic findings

GA and BBW were significantly lower in the cholestasis group (28.6±2.4 vs. 30.0±2.9, 1,056.7±224.5 vs. 1,210.8±2,443.7, $p<0.05$). The durations of oxygen supply and mechanical ventilator care were significantly longer in the cholestasis group (73.7±47.4 vs. 46.2±45.0, 36.5±35.0 vs. 19.0±31.4, $p<0.05$). The prevalence rate of PDA, the rate of medication treatment for PDA, and the frequency of dexamethasone administration were higher in the cholestasis group (78.3% vs. 51.1%, 69.6% vs. 46.1%, 52.2% vs. 31.2%, $p<0.05$). The duration of antimicrobial administration and the length of hospitalization were longer in the cholestasis group (27.1±19.8 vs. 13.9±15.7, 101.4±34.6 vs. 72.5±39.9, $p<0.05$). To eliminate the influences of GA and BBW, we used an ANCOVA test to regulate the statistically significant results. After this regulation, only the duration of antimicrobial administration was significantly associated with PNAC (Table 1).

Factors associated with PN, EF, and nutritional data

The durations of administration of PN, amino acid, lipid, and trace elements were longer in the cholestasis group (55.0±23.8 vs. 29.0±16.3, 55.0±23.8 vs. 29.0±16.3, 48.0±20.4 vs. 24.3±15.9, 43.6±18.3 vs. 27.3±16.4, $p<0.05$). In this group, delayed time to initiate feeding, delayed time to near-full EN, delayed time to full EN, and a longer duration of fasting were also observed (6.2±6.7 vs. 3.2±3.4, 52.2±20.3 vs. 29.9±14.8, 56.7±24.4 vs. 32.8±16.1, 18.7±11.0 vs. 6.8±6.8, $p<0.05$). Other significant differences of the cholestasis group were higher PN calories at the second and fourth weeks of life; lower EN calories at the first, second, and fourth weeks of life; and higher intravenous amino acid and lipid administration at the fourth week of life (104.8±24.5 vs. 89.4±41.2, 102.0±40.4 vs. 45.0±53.1, 8.5±10.8 vs. 19.3±21.3, 11.7±16.2 vs. 40.1±36.2, 29.6±30.8 vs. 82.6±41.0, 3.0±1.1 vs. 1.6±1.7, 2.8±1.2 vs. 1.1±1.5, $p<0.05$). After adjusting for the influences of GA and BBW by using the ANCOVA test, all of the above-mentioned statistical results were significantly associated with PNAC, except for the duration of trace element administration (Tables 2 and 3).

Table 1. Differences of Perinatal Characteristics and Neonatal Demographic Findings between the Cholestasis Group and the Non-Cholestasis Group

Variable	Cholestasis (n=46)	Non-cholestasis (n=141)	*p*-value*	*p'*-value†
Maternal PIH	9 (19.6)	25 (17.7)	NS	NS
Maternal DM	0 (0.0)	5 (3.5)	NS	NS
PPROM	23 (50.0)	68 (48.2)	NS	NS
Antenatal steroids	36 (78.3)	82 (58.2)	NS	NS
Male	24 (52.2)	67 (47.5)	NS	NS
GA (week)	28.6±2.4	30.0±2.9	0.001	-
BBW (g)	1,056.7±224.5	1,210.8±2443.7	0.000	-
Apgar score at 1 minute	4.4±1.8	4.2±1.9	NS	NS
Apgar score at 5 minute	6.4±1.7	6.4±1.6	NS	NS
SGA	7 (15.2)	27 (19.1)	NS	NS
RDS	35 (76.1)	87 (61.7)	NS	NS
Duration of oxygen supply (day)	73.7±47.4	46.2±45.0	0.001	NS
Duration of ventilator care (day)	36.5±35.0	19.0±31.4	0.003	NS
PDA	36 (78.3)	72 (51.1)	0.001	NS
Indomethacin or Ibuprofen use	32 (69.6)	65 (46.1)	0.007	NS
Fluconazole prophylaxis	6 (13.0)	11 (7.8)	NS	NS
Duration of antimicrobials (day)	27.1±19.8	13.9±15.7	0.000	0.016
Dexamethasone use	24 (52.2)	44 (31.2)	0.013	NS
Congenital hypothyroidism	5 (10.9)	8 (5.7)	NS	NS
Length of hospitalization (day)	101.4±34.6	72.5±39.9	0.000	NS
Return to birth weight (postnatal day)	13.5±7.5	12.4±5.3	NS	NS

Values are presented as number (%) or mean±standard deviation.
*Statistics were analyzed by *t*-test and chi-square test. †ANCOVA (analysis of covariance) test to adjust for GA and birth body weight. PIH: pregnancy-induced hypertension, DM: diabetes mellitus, PPROM: premature preterm rupture of membrane, GA: gestational age, BBW: birth body weight, SGA: small for GA, RDS: respiratory distress syndrome, PDA: patent ductus arteriosus, NS: nonspecific.

Table 2. Comparison of Factors Associated with Parenteral Nutrition and Enteral Nutrition between the Cholestasis Group and the Non-Cholestasis Group

Variable	Cholestasis (n=46)	Non-cholestasis (n=141)	*p*-value*	*p'*-value†
PN duration (day)	55.0±23.8	29.0±16.3	0.000	0.000
Amino acid duration (day)	55.0±23.8	29.0±16.3	0.000	0.000
Lipid start (postnatal day)	2.4±1.0	2.8±1.3	NS	NS
Lipid duration (day)	48.0±20.4	24.3±15.9	0.000	0.001
Trace elements duration (day)	43.6±18.3	27.3±16.4	0.000	NS
Time to initiate feeding (postnatal day)	6.2±6.7	3.2±3.4	0.006	0.000
Time to near full EN (postnatal day)	52.2±20.3	29.9±14.8	0.000	0.000
Time to full EN (postnatal day)	56.7±24.4	32.8±16.1	0.000	0.000
Duration of fasting (day)	18.7±11.0	6.8±6.8	0.000	0.000

Values are presented as mean±standard deviation.
*Statistics were analyzed by *t*-test. †ANCOVA (analysis of covariance) test to adjust for gestational age and birth body weight. PN: parenteral nutrition, EN: enteral nutrition, NS: nonspecific.

Postnatal complications

The prevalence of NEC, BPD, and ROP, and the rates of GI operation and skeletal fracture were higher in the cholestasis group (15.2% vs. 0.7%, 60.9% vs. 42.6%, 30.4% vs. 12.1%, 28.3% vs. 3.5%, 6.5% vs. 0.0%, $p<0.05$). NEC, GI operation, and skeletal fracture were significantly associated with PNAC after adjustment for GA and BBW (Table 4).

Table 3. Comparison of Nutritional Data between the Cholestasis Group and the Non-Cholestasis Group

Variable	Cholestasis (n=46)	Non-cholestasis (n=141)	*p*-value*	*p'*-value†
PN calories (kcal/kg/day)				
1st week	90.5±22.2	92.4±21.4	NS	NS
2nd week	104.8±24.5	89.4±41.2	0.018	0.045
4th week	102.0±40.4	45.0±53.1	0.000	0.011
EN calories (kcal/kg/day)				
1st week	8.5±10.8	19.3±21.3	0.001	0.004
2nd week	11.7±16.2	40.1±36.2	0.000	0.001
4th week	29.6±30.8	82.6±41.0	0.000	0.039
Intravenous amino acid (g/kg/day)				
1st week	2.7±0.8	2.8±0.8	NS	NS
2nd week	3.1±0.8	3.0±1.5	NS	NS
4th week	3.0±1.1	1.6±1.7	0.000	0.000
Intravenous lipid (g/kg/day)				
1st week	2.4±0.8	2.2±0.9	NS	NS
2nd week	2.9±0.8	2.4±1.4	NS	NS
4th week	2.8±1.2	1.1±1.5	0.000	0.021

Values are presented as mean±standard deviation.
*Statistics were analyzed by *t*-test. †ANCOVA (analysis of covariance) test to adjust for gestational age and birth body weight. PN: parenteral nutrition, EN: enteral nutrition, NS: nonspecific.

Table 4. Comparison of Postnatal Complications between the Cholestasis Group and the Non-Cholestasis Group

Variable	Cholestasis (n=46)	Non-cholestasis (n=141)	*p*-value*	*p'*-value†
NEC (≥stage 2)	7 (15.2)	1 (0.7)	0.000	0.000
GI operation	13 (28.3)	5 (3.5)	0.000	0.000
Sepsis	13 (28.3)	22 (15.6)	NS	NS
BPD	28 (60.9)	60 (42.6)	0.041	NS
IVH (≥grade 3)	5 (10.9)	6 (4.3)	NS	NS
ROP (≥stage plus)	14 (30.4)	17 (12.1)	0.006	NS
PVL	3 (6.5)	10 (7.1)	NS	NS
Neurosensory hearing loss	0 (0.0)	5 (3.5)	NS	NS
Skeletal fracture	3 (6.5)	0 (0.0)	0.001	0.001

Values are presented as number (%).
*Statistics were analyzed by chi-square test. †ANCOVA (analysis of covariance) test to adjust for gestational age and birth body weight. NEC: necrotizing enterocolitis, GI: gastrointestinal, BPD: bronchopulmonary dysplasia, IVH: intravascular hemorrhage, ROP: retinopathy of prematurity, PVL: preventricular leukomalacia, NS: nonspecific.

Laboratory findings

The maximum levels of alanine aminotransferase (202.4±176.0, $p<0.05$), aspartate aminotransferase (252.3±192.7, $p<0.05$), alkaline phosphatase (2,297.1±697.4, $p<0.05$), and total bilirubin (10.6±4.1, $p<0.05$) were higher in the cholestasis group than in the control group. The maximum direct bilirubin (DB) level of the cholestasis group was 6.8 mg/dL. The time to cholestasis development was 52.4 days; the mean time to peak DB was 70.5 days. Cholestasis persisted for 63.3 days; the peak prothrombin time international normalized ratio was 1.4 (Table 5).

Independent predictive factors

A multivariate logistic regression analysis was performed on the statistically significant results to evaluate the independent predictive factors of PNAC. The results of this analysis suggested that the factors that significantly increased the risk of PNAC were

Table 5. Comparison of Laboratory Findings between the Cholestasis Group and the Non-Cholestasis Group

Variable	Cholestasis (n=46)	Non-cholestasis (n=141)	*p*-value*
ALT (IU/L)	202.4±176.0	37.0±34.8	0.000
AST (IU/L)	252.3±192.7	74.9±131.6	0.000
ALP (IU/L)	2,297.1±697.4	1,625.3±748.9	0.000
TB (mg/dL)	10.6±4.1	8.7±2.7	0.004
Peak DB (mg/dL)	6.8		
Time to cholestasis (day)	52.4		
Time to peak DB (day)	70.5		
Duration of cholestasis (day)	63.3		
Peak PT INR	1.4		

Values are presented as mean±standard deviation or mean only.
*Statistics were analyzed by *t*-test.
ALT: alanine aminotransferase, AST: aspartate aminotrasferase, ALP: alkaline phosphatase, TB: total bilirubin, DB: direct bilirubin, PT INR: prothrombin time international normalized ratio.

Table 6. Logistic Regression Analysis for the Predictive Factors of Parenteral Nutrition-Associated Cholestasis

Variable	Odds ratio	95% CI		*p*-value*
		Lower	Upper	
Duration of antimicrobials (day)	4.49	4.42	4.58	0.000
PN duration (day)	2.68	2.41	3.00	0.000
Amino acid duration (day)	2.68	2.41	3.00	0.000
Lipid duration (day)	1.99	1.43	4.45	NS
Time to initiate feeding (postnatal day)	1.64	1.22	3.61	NS
Time to near full EN (postnatal day)	1.21	0.51	2.73	NS
Time to full EN (postnatal day)	1.97	0.71	4.06	NS
Duration of fasting (day)	2.89	2.43	3.37	0.000
PN calories (kcal/kg/day)				
2nd week	1.14	0.44	2.81	NS
4th week	1.27	0.41	3.97	NS
EN calories (kcal/kg/day)				
1st week	0.57	0.22	1.98	NS
2nd week	0.79	0.21	2.97	NS
4th week	0.56	0.36	1.82	NS
Intravenous amino acid (g/kg/day)				
4th week	1.21	0.23	3.62	NS
Intravenous lipid (g/kg/day)				
4th week	1.12	0.37	3.32	NS
NEC (≥stage 2)	2.40	2.16	2.83	0.000
GI operation (%)	2.19	2.03	2.58	0.000
Skeletal facture (%)	1.11	0.20	2.78	NS

*Statistics were analyzed by logistic regression analysis.
CI: confidence interval, PN: parenteral nutrition, EN: enteral nutrition, NEC: necrotizing enterocolitis, GI: gastrointestinal, NS: nonspecific.

the duration of antimicrobial use (odds ratio [OR] 4.49, 95% confidence interval [95% CI] 4.42-4.58), PN (OR 2.68, 95% CI 2.41-3.00), amino acids (OR 2.68, 95% CI 2.41-3.00), and fasting (OR 2.89, 95% CI 2.43-3.37), the occurrence of NEC (OR 2.40, 95% CI 2.16-2.83), and GI operation (OR 2.19, 95% CI 2.03-2.58) (Table 6).

DISCUSSION

Although PNAC has been studied for a long time, its definite etiology is still unknown. The pathogenesis is considered to be multifactorial. Owing to a combination of immature bile acid synthetic and transport processes, bile flow in newborns, especially preterm infants, is low. The development of hepatocyte transporters is not sufficiently complete and the plasma bile acid levels in these infants are higher than the normal adult range; thus, the immaturity of bile flow and retention promotes the development of cholestasis [3]. Lack of EN also plays a role in the development of cholestasis owing to reduced secretion of intestinal hormones and destruction of the enterohepatic circulation [4]. Preterm infants are also vulnerable to infections and intestinal problems such as NEC; moreover, these infants need to receive prolonged PN which can aggravate cholestasis [5]. Some studies suggested that PN components, such as amino acid, cause cholestasis. In 2006, Choi et al. [6] reported that the frequency of PNAC in VLBWIs could be decreased by adjusting the composition of amino acid mixtures in PN. Also, ω-6 fatty acids, phytosterols in soybean oil, and trace elements such as copper and manganese, acting as toxicants, can lead to cholestasis [7,8].

In our study, the duration of antimicrobial administration was an independent risk factor for PNAC. Almost all VLBWIs in our NICU received antimicrobials such as ampicillin, penicillin, aminoglycoside, cephalosporin, vancomycin, meropenem, fluconazole, amphotericin B, and caspofungin. Brown et al. suggested that antimicrobials were an important cause of drug-induced liver injury due to hepatocellular, cholestatic, or mixed reactions [9]. Especially, the cholestasis condition resulted from interference with hepatocyte canalicular efflux systems for bile salts, organic anions, and phospholipids due to the inhibition of the bile salt export pump [10]. The incidence of drug-induced liver injury is low and the prognosis is generally good; however, VLBWIs are exposed to numerous risk factors of cholestasis, such as prolonged PN and lack of EN, sepsis, hypoxia, and their immature biliary excretion [4]. To reduce antimicrobial-related cholestasis, antimicrobials should be used properly and quickly stopped when the infection is solved.

Previous studies reported that, in addition to prolonged PN, sepsis should be considered another cause of cholestasis. The mechanism of sepsis-associated cholestasis was direct hepatotoxicity due to endotoxin production by bacteria overgrowing in the intestine, especially, gram-negative bacteria [11]. By contrast, gram-positive bacteremia, such as streptococcal and staphylococcal infections, was not common cause of liver abnormalities in neonates. However, infection with Listeria monocytogenes always provoked liver abnormalities, such as hepatitis and cholestasis [12]. Also, who had intestinal failure and received prolonged PN could have complications, such as catheter related sepsis which could had intestinal bacterial overgrowth may cause bacterial translocation and following cholestasis [13]. However, in our study, sepsis was not a risk factor of PNAC. This was probably, the greater part of sepsis were gram-positive bacterial infections, such as staphylococcus aureus and staphylococcus epidermidis in our NICU.

The use of fluconazole prophylaxis has been an empirical treatment method for VLBWIs in many NICUs; however, due to the lack of long-term outcomes, potential toxicities, including hepatotoxicity, remain a concern. Aghai et al. [14] reported that, when used in appropriate dose and for a targeted short-term period, fluconazole prophylaxis was effective in preventing fungal infection and was not associated with an increased incidence of cholestasis. Congruently with these findings, Kaufman et al. [15] reported, frequency of cholestasis was no difference between preterm infants who had fluconazole prophylaxis and controls. Similarly to these findings, fluconazole prophylaxis did not increase the incidence of cholestasis in our study.

In numerous previous studies, the duration of PN was associated with PNAC [16-18]. Prolonged supply of PN leads to hyperinsulinemia, which converts glucose into fat, resulting in hepatic fatty infiltration and leading to cholestasis [19]. In our NICU, we

started PN and amino acid at the day of birth; therefore, the duration of amino acid administration corresponded to the duration of PN. Similarly to the other studies, the duration of PN was independently associated with PNAC in our study.

According to a recent study, minimal PN caloric intake of 60 kcal/kg/d should be given to prevent catabolism in preterm infants and PN calories could be increasing to up to 90-108 kcal/kg/d which was appropriated to maintain body temperature [20]. Messing et al. [21] reported that excessive parenteral administration of protein, lipid, and calories induce PNAC. Also, Lloyd et al. [22] reported that increasing parenteral energy is a risk factor of cholestasis. Excessive calories due to carbohydrate and lipid overload could lead to hepatic steatosis and cholestasis [5]. Furthermore, increasing the intravenous amino acid causes direct damage to the hepatic cellular canalicular membrane, leading to cholestasis [23]. However, our data analysis demonstrated that the PN calories and the amount of amino acid and lipid were not independent predictive factors of PNAC. As in Kang et al., the amount of amino acid was not an independent risk factor of cholestasis in this study [24].

There is no confirmed link between amino acid components of PN and cholestasis. Several studies reported that some amino acids, such as tryptophan, methionine, and phenylalanine, cause hepatocellular toxicity; however, taurine increases bile acid excretion and glutamine prevents gut atrophy [25,26]. We did not investigate the amino acid composition, although our NICU used amino acids (10% Primene), including taurine and glutamine. Further research is needed to evaluate the relation between amino acid components and cholestasis.

Early EN in preterm infants can lead to early full EN. Also, early EN promotes maturation of bowel motility, prevents intestinal atrophy, and reduces the PN duration and the incidence of cholestasis [27]. For these reasons, early EN and reduction of the time to full EN are excellent strategies for preventing PNAC in VLBWIs. However, congruently with Loomis et al. [28], the time to initiate EN and time to full EN were not established to be statistically independent risk factors of PNAC in our study.

Long-term fasting has been suggested to be an important risk factor of cholestasis. Lack of EN can reduce the secretion of intestinal hormones, such as gastrin and cholecystokinin, and disturb bile acid secretions. Also, lack of EN can reduce the enterohepatic circulation of bile acid [4]. Moreover, decreased enterohepatic circulation leads to bacterial overgrowth, which, in turn, can inhibit bile acid secretion, leading to cholestasis [29]. Also, lack of EN can lead to prolonged PN and its associated complications, such as cholestasis. The length of enteral rest was also an independent risk factor of PNAC in our study.

Christensen et al. [17] reported that NICU patients who had undergone intestinal surgery because of NEC, gastroschisis, or jejunal atresia had a high risk of PNAC. We also found an association between GI operation and PNAC, which seems to be related to the prolonged lack of EN after GI operation. Similarly to our results, a previous study suggested that NEC altered the hepatobiliary function, leading to biliary stasis. Degenerated hepatocytes could make the liver vulnerable to damage from PN; thus, NEC contributes to PNAC [30].

In conclusion, longer duration of antimicrobial administration, longer duration of PN, long-term lack of EN, occurrence of NEC, and GI operation were established to be the risk factors of PNAC in VLBWIs. In other words, aggressive EN and shortened duration of PN, as well as minimal use of antimicrobials, will be helpful in preventing PNAC.

However, this study has some limitations, including its being a short-term study (5 years, from the beginning of the PN) and its retrospective nature. Also, postnatal complications can be greater in the cholestasis group, as these infants are sicker than those in the non-cholestatic group. Consequently, a longer-term study and a study that would compare infants with a similar health status are needed to more comprehensively evaluate the risk factors of PNAC in VLBWIs.

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