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Two parenteral amino acid solutions and plasma levels of amino acids in the neonate: A randomized trial

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ABSTRACT

Objective: In neonates on total parenteral nutrition (TPN), amino acids may be a risk factor for developing total parenteral nutrition–associated cholestasis (TPNAC). We aimed, first, to compare methionine, cysteine, and taurine plasma levels between neonates on TPN who were receiving an intravenous amino acid solution based on a breast milk aminogram and those on an intravenous solution of pediatric amino acids based on an umbilical cord aminogram, and second, to determine the frequency of TPNAC.
Methods: A double-blind randomized controlled trial was conducted. Ninety-four neonates with a birthweight of 1000 g or more and a gestational age of 30 wk or older were admitted and enrolled. Blood samples were obtained at 0, 7, and 14 d of TPN, and plasma amino acid concentrations were determined by ultra-high-resolution liquid chromatography. Continuous variables were compared using the Wilcoxon rank-sum test or Student's *t* test; categorical variables were compared using the Fisher exact test.
Results: Thirty-five neonates completed the study (Primene, $n = 14$; TrophAmine, $n = 21$). On day 14, methionine plasma concentrations were significantly lower in the Primene group than in the TrophAmine group (27 μmol/L versus 32.9 μmol/L, $P = 0.044$); the taurine concentration was significantly higher in the same group (72.4 μmol/L versus 45.3 μmol/L, $P < 0.0001$). There were no differences in TPNAC incidence.
Conclusions: Administering an intravenous solution of pediatric amino acids based on the umbilical cord aminogram yielded a higher taurine and lower methionine plasma concentration than did administering a similar solution based on the breast milk aminogram.

Introduction

The prolonged use of total parenteral nutrition (TPN) is associated with the development of several complications, among which cholestasis is one of the most common (7.4–84%) and can lead to terminal liver failure and death [1–3].

Patient-dependent risk factors include the degree of liver maturation (prematurity), low birth weight, sepsis, necrotizing enterocolitis, small-intestine bacterial overgrowth, abdominal surgery with prolonged maintenance of stomas interrupting the enterohepatic circulation, and lack of enteral intake [4]. Parenteral nutrition–related risk factors include excessive caloric intake, type of amino acid and lipid solutions (contamination with phytosterols), carnitine deficiency, osmolarity, polysorbates, and prolonged TPN use. In addition, the possible toxicity of specific nutrients added to TPN, such as copper, manganese, and aluminum, also lead to the development of liver dysfunction [2,5–7].

Clinical Trial Registration: Total Parenteral Nutrition Associated Cholestasis (TPNAC) and Plasma Amino Acid Levels in Neonates, NCT 01062724; ClinicalTrials.gov.

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Regarding the composition of TPN, amino acids have been proposed as a risk factor. The composition of intravenous amino acid solutions has been suggested to alter hepatic function through its effect on the transport and conjugation of bile acids [1,8]. Because of the immaturity of hepatic degradation pathways in the newborn, amino acid solutions may increase the plasma methionine concentration [9,10]. In addition, taurine deficiency may play a role in the development of total parenteral nutrition–associated cholestasis (TPNAC) in neonates because taurine serves to solubilize bile salts and is therefore necessary for adequate biliary secretion, ileal reabsorption, and protection against lithocholic acid toxicity [2,11]. Currently, amino acid solutions used in pediatrics are designed to reproduce the aminogram of either umbilical cord plasma or breast milk [12–15]. However, these solutions differ in the content of methionine, cysteine, and taurine, which are amino acids associated with the presence of this complication [15–20]. To the best of our knowledge, there are no randomized controlled clinical trials comparing the plasma concentrations of methionine, cysteine, and taurine in neonates and their relation to the presence of TPNAC when amino acid solutions such as Primene and TrophAmine are administered. Therefore we conducted a clinical trial with the primary aim of comparing the plasma levels of methionine, cysteine, and taurine in neonates on TPN receiving either an intravenous solution of pediatric amino acids based on the umbilical cord aminogram (10% Primene) or an intravenous amino acid solution based on the breast milk aminogram (10% TrophAmine), with the secondary aim of identifying whether amino acid serum concentrations may be a predisposing factor related to TPNAC.

Materials and methods

Study design

We conducted a double-blind randomized controlled clinical trial. Approval was obtained from the Research and Ethics Committee of the Mexican Institute of Social Security in Mexico City (Approval no. 2009-785-080), and all parents or legal guardians of the neonates gave their written informed consent after the procedures had been explained. This trial is registered at clinicaltrials.gov (NCT 01062724).

Eligibility criteria

Our study included newborns weighing 1000 g or more, with a gestational age of 30 wk or older, who were admitted to the Neonatal Intensive Care Unit of the Pediatric Hospital in Mexico City (Mexican Institute of Social Security, IMSS) and had a pathologic diagnosis requiring TPN support (necrotizing enterocolitis, intestinal atresia, short-bowel syndrome). Participants also had baseline direct bilirubin levels of less than 1 mg/dL and normal liver function tests for their age before the initiation of TPN and were expected to be TPN dependent for 14 d or more. We excluded neonates before exposure to TPN if they had acute renal failure, congenital liver disease, end-stage liver disease, liver damage secondary to viral or bacterial infections, or liver damage secondary to drugs.

Recruitment, allocation, and intervention

Neonates were randomly assigned to one of two different intravenous amino acid solution groups by a computer-generated list of random numbers using software for parallel groups (Random Allocation Software, http://www.msaghaei.com/Softwares/dnld/RA.zip) [21]. The allocation was carried out by balanced blocks of six neonates. The parents, physicians, researchers, and nutritionists were blinded to the treatment allocation for the duration of the study. The unblinded specialist nurse of the parenteral nutrition service supervised the random allocation and assigned the amino acid solution according to the corresponding group. Investigators were blinded for the allocation until after initial statistical analyses were performed.

Intervention

Parenteral Nutrition Support

TPN was administered according to standardized Guidelines on Pediatric Parenteral Nutrition 2005 [22] as follows: glucose infusion was begun at 4 to 8 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} on the first day of TPN to a maximum of 11 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}. Protein administration began at a minimum amino acid intake of 1.5 g $\cdot$ kg^{-1} $\cdot$ d^{-1} and advanced 1.0 g $\cdot$ kg^{-1} $\cdot$ d^{-1} to a maximum of 3.0 g $\cdot$ kg^{-1} $\cdot$ d^{-1} in term neonates or 4.0 g $\cdot$ kg^{-1} $\cdot$ d^{-1} in preterm infants. Lipid administration (20% Lipofundin MCT/LCT, B Braun, Melsungen, Germany) began at 1.0 g $\cdot$ kg^{-1} $\cdot$ d^{-1} on the first day of TPN and was advanced by 0.5 to 1.0 g $\cdot$ kg^{-1} $\cdot$ d^{-1} to a maximum of 3 g $\cdot$ kg^{-1} $\cdot$ d^{-1}. In addition, energy intake (kcal/d and kcal $\cdot$ kg^{-1} $\cdot$ d^{-1}), lipids, protein, and carbohydrates were documented at the beginning and during parenteral nutrition. The components used for preparation of TPN did not change within the study period. In this study, two different intravenous amino acid solutions were administered: one of the infant groups received an intravenous amino acid solution based on the breast milk aminogram (10% TrophAmine, Kendall McGaw Laboratories, Irvine, CA, USA), and the other group received an intravenous solution of pediatric amino acids based on the umbilical cord aminogram (10% Primene Clintec Benelux NV, Brussels, Belgium) (Table 1). None of the infants received supplemental cysteine or taurine to the parenteral nutrition solutions.

Sample size

We hypothesized that neonates with TPN receiving an intravenous solution of pediatric amino acids based on the umbilical cord aminogram would have a decreased incidence of cholestasis and plasma concentration of methionine and higher plasma concentration of taurine. Sample size was calculated based on circulating plasma methionine levels at day 14 of TPN according to studies by Bulbul et al. [23] and Heird et al. [14], with 80% sample power and a 5% significance level. We estimated that with a minimum of 8 patients and a 30% dropout rate, a total sample of 12 participants in each group would allow the detection of differences in the plasma concentrations of amino acids.

Analytical methods

Blood sample

Blood samples were obtained from peripheral veins at specific time points: just before starting the parenteral nutrition and again at days 7 and 14 of TPN. All samples were collected into heparinized tubes and immediately separated. Plasma aliquots were stored at −80°C until analysis. The volume of the blood samples did not exceed the limit given by the Research and Ethics Committees of the Mexican Institute of Social Security in Mexico City (<1% of the estimated total blood volume), and whenever a blood sample was obtained, blood parameter testing was grouped to avoid multiple punctures. Initially the study was designed to evaluate

Table 1
Amino acid composition of TrophAmine and Primene

	10% Primene aminogram umbilical cord	10% Troph Amine aminogram breast milk
Essential amino acids (g/100 mL)		
L-Isoleucine	0.670	0.820
L-Leucine	1.000	1.40
L-Valine	0.760	0.780
L-Lysine	1.100	0.820
L-Methionine	0.240	0.340
L-Phenylalanine	0.420	0.480
L-Threonine	0.370	0.420
L-Tryptophan	0.200	0.200
L-Histidine	0.380	0.480
Conditionally essential (g/100 mL)		
L-Tyrosine	0.045	0.24
L-Cysteine	0.189	< 0.016
L-Arginine	0.840	1.2
L-Glycine	0.400	0.36
L-Proline	0.300	0.68
Nonessential amino acids (g/100 mL)		
L-Alanine	0.800	0.540
L-Aspartic-acid	0.600	0.320
L-Asparagine	—	—
L-Glutamic acid	1.000	0.500
L-Serine	0.400	0.380
L-Taurine	0.060	0.025
L-Ornithine	0.249	—
Osmolarity (mOsmol/L)	780	875
Total amino acids (100 g/L)		
Total nitrogen (g/L)	15	15.5
Chlorides (mmol/L)	15.6	—
Approximate pH	5.5	5.5

Adapted from Wright et al. [15] and Sáenz de Pipaón [31].

changes in the plasma concentration of these amino acids and cholestasis frequency at days 21 and 28; unfortunately, this evaluation was not possible owing to the inevitable number of dropouts.

Plasma amino acid analysis

Plasma amino acid concentrations were determined using ultra-high-resolution liquid chromatography (Acquity UPLC, Ultraperformance LC, Waters Corp., Milford, MA, USA) [24–26]. Retention times and peak areas were calculated automatically. Standard solutions of known enrichments were run simultaneously.

Cholestasis

Cholestasis was defined as serum direct bilirubin greater than 2 mg/dL for 14 or more consecutive days. Analyses of bilirubin with fractions were performed at days 7 and 14 of TPN. Bilirubin concentration was measured in a Cobas 6000 (Cobas c-501) Hitachi analyzer (Roche Diagnostics, Indianapolis, IN, USA). During the study, other biomarkers of hepatic function such as transaminases were regularly checked.

Statistical analysis

The data were analyzed using the SPSS Version 21.0 for Windows (IBM Corp., Armonk, NY, USA). Data are expressed as the median (minimum, maximum), and categorical variables are presented as number (percentage). Variables without normal distribution were compared using the Wilcoxon rank-sum test, whereas categorical variables were compared using Fisher exact test and the Pearson χ^2 test as appropriate. Statistical significance was defined a priori as a P value < 0.05. Associations between the interventions of two amino acid solutions were analyzed using a multivariate logistic regression model and expressed as odds ratios with a 95% confidence interval. The potentially relevant confounders in the multivariate logistic regression analysis were age at start of TPN (days), amino acid solution group, plasma amino acid concentration, and enteral feeding. The analysis was by original assigned groups.

Results

Over a 4.5-y period (May 2011–November 2015), a total of 113 patients were identified, and 94 were enrolled. Forty-five patients were randomly assigned to the TrophAmine group and 49 to the Primene group. A total of 24 out of 45 patients in the TrophAmine group and 31 of 49 in the Primene group discontinued TPN administration. The decision to suspend TPN was indicated by the treating physician and the parenteral nutrition specialist according to the adverse event or complication presented (Fig. 1).

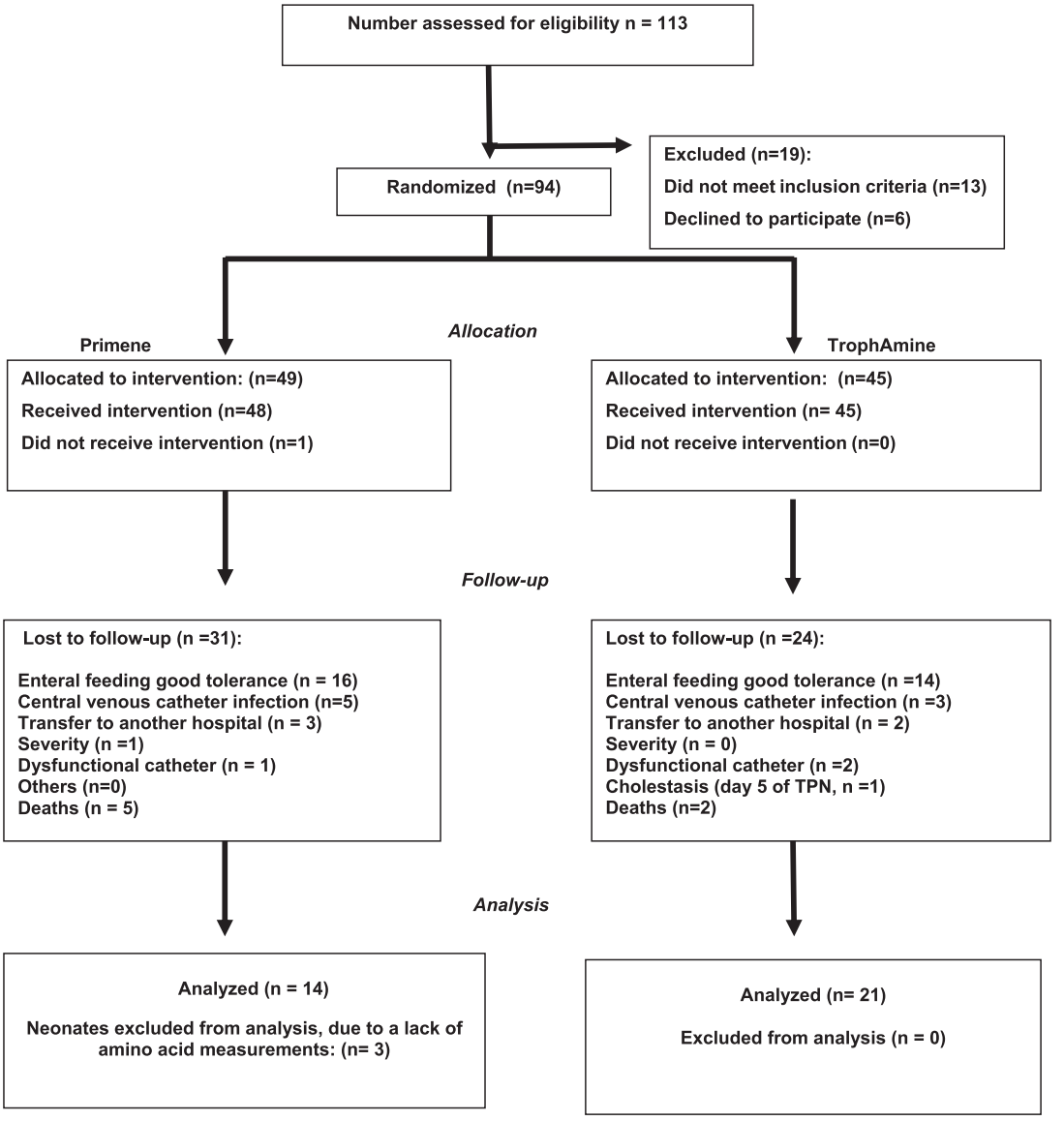

Figure 1. CONSORT diagram showing the flow of participants through each stage of a randomized trial.

Table 2
Characteristics of neonates at baseline time

Parameter	10% Primene $n = 14$	10% TrophAmine $n = 21$	P value
Sex*			
Male	8 (57)	14 (67)	0.41
GE (wk)†	37 (28, 40)	37 (28, 41)	0.83
CGE (wk)†	37.3 (30.1, 40.5)	37.2 (28.6, 42)	0.91
Preterm infants*	5 (36)	8 (38)	0.58
Birthweight (g)†	2,225 (1,000; 3,400)	2,330 (1,000; 3,970)	0.77
Birth length (cm)†	46 (34,51)	46 (38, 51)	0.58
Head circumference (cm)†	32.8 (26,37)	33.5 (25, 38.5)	0.67
Apgar (1 min)†	7 (2, 8)	7 (3, 9)	0.72
Apgar (5 min)†	8 (7, 9)	8 (5, 9)	0.70
Diagnosis*:			
Gastroschisis	5 (35.7)	4 (19.4)	NS
Intestinal atresia	3 (21.4)	4 (19.0)	
Esophageal atresia	3 (21.4)	3 (14.3)	
Diaphragmatic hernia	2 (14.2)	2 (9.52)	
Anorectal malformation	0	3 (14.3)	
Congenital heart disease	0	2 (9.5)	
Necrotizing enterocolitis	1 (7.1)	2 (9.5)	
Neonatal asphyxia	0	1 (4.8)	

CGE, corrected gestational age; GE, gestational age; NS, nonsignificant.
Comparison between groups with Fisher exact test; Comparison between groups with the Mann-Whitney U test.
*Number (%).
†Median (minimum, maximum).

Table 3
Characteristics of parenteral and enteral nutrition during the study

	10% Primene $n = 14$	10% TrophAmine $n = 21$	P value
Parenteral nutrition			
TPN time (d)*	14 (13, 14)	14 (12, 14)	0.90
Age at start of TPN (d)*	3 (1, 15)	4 (1, 31)	0.87
Daily parenteral caloric intake (kcal/d)†	211 ± 48	205 ± 66	0.93
Daily parenteral caloric intake ($kcal \cdot kg^{-1} \cdot d^{-1}$)†	89 ± 6.9	84 ± 7.8	0.052
Daily parenteral protein intake ($g \cdot kg^{-1} \cdot d^{-1}$)†	2.7 ± 0.19	2.8 ± 0.47	0.38
Daily parenteral lipid intake ($g \cdot kg^{-1} \cdot d^{-1}$)†	2.5 ± 0.30	2.4 ± 0.40	0.88
Daily parenteral carbohydrate intake ($g \cdot kg^{-1} \cdot d^{-1}$)†	13.2 ± 1.1	12.9 ± 0.84	0.28
Enteral nutrition	***n* = 4**	***n* = 11**	
Day of first oral feeding (d)*	10 (7, 14)	7 (1, 13)	—
Baseline age (d)*	12 (10, 18)	14 (7, 37)	—
Intake $kcal \cdot kg^{-1} \cdot d^{-1}$*	22.8 (17, 24)	38 (8, 101)	—

TPN, total parenteral nutrition.
Data presented are median (interquartile range)* and in the case of continuous variables with normal distribution as the mean ± standard deviation.†
*Comparison between groups with the Mann-Whitney U test.
†Comparison between groups with Student's t test.

Baseline characteristics

The baseline characteristics of the neonates analyzed are presented in Table 2. There were no differences at the beginning of TPN between neonates who received Primene and those who received TrophAmine. The most common diagnosis for the two treatment groups was gastroschisis (Primene 35.7%, TrophAmine 19.4%), followed by intestinal atresia (Primene 21.4%, TrophAmine 19%) and esophageal atresia (Primene 21.4%, TrophAmine 14.3%).

Characteristics of the parenteral and enteral nutrition

There were no significant differences in average energy intake, lipids, protein, and carbohydrates from parenteral nutrition between neonates who received Primene and those who received TrophAmine. The age of the neonates at the time of starting the enteral nutrition fluctuated between 10 and 18 d for the Primene group and between 7 and 37 d for the TrophAmine group (Table 3).

Analysis of amino acids between groups

Analysis of amino acid concentrations indicated that the groups were comparable at the beginning of TPN (Table 4). On day 14, methionine plasma concentrations were significantly lower in the Primene group than in the TrophAmine group (27 μmol/L versus 32.9 μmol/L, $P = 0.044$); conversely, the taurine plasma concentration was significantly higher in the same group (72.4 μmol/L versus 45.3 μmol/L, $P < 0.0001$), whereas the cysteine concentration was not different (44.8 μmol/L versus 62.8 μmol/L; $P = 0.359$).

Analysis of intragroup amino acid concentration

In both groups the plasma concentration of methionine increased significantly at day 14 of TPN; in the infants assigned to the TrophAmine group, the plasma concentration increased by 110%; and in the infants assigned to the Primene group, the increase was 66% and did not reach statistical significance. The plasma taurine concentration increased by 61% in the Primene group and just 7.5% in the TrophAmine group.

Logistic regression models were constructed using age at start of TPN, the amino acid solution, and the concentration of plasma amino acids at day 14 of TPN. We found that age at start of TPN, as reported previously, is predictive of cholestasis, whereas methionine concentration was only marginally significant (model 2), suggesting that this amino acid could be an independent predictor of cholestasis (Table 5).

Cholestasis incidence

The incidence of TPNAC was similar in both groups ($P = 0.47$). Of the nine patients with TPNAC, 3 of 14 (21.4%) received Primene solution and 6 of 21 (28.6%) received TrophAmine. We found that the maximum value (11.8 mg/dL) of direct bilirubin was higher in

Table 4
Plasma amino acid concentrations during study

Amino acid	10% Primene $n = 14$ μmol/L	10% TrophAmine $n = 21$ μmol/L	P value
Methionine			
Baseline	14.5 (5.6, 38.5)	23 (8.6, 51.2)	0.175
Day 7	22.9 (10.8, 62)*	25 (10.8, 63.2)*	0.583
Day 14	27 (9.8, 61.1)*	32.9 (17.2, 125.3)*,†	0.044
Cysteine			
Baseline	21.2 (4.3, 287)	23.1 (3.4, 242.5)	0.829
Day 7	37.8 (5.1, 193.6)	45.8 (3.4, 213.2)*	0.630
Day 14	44.8 (6.6, 175.4)	62.8 (3.0, 263.6)*,†	0.359
Taurine			
Baseline	39.3 (16.2, 97.5)	41.2 (14.6, 137.3)	0.934
Day 7	67.9 (30.3, 123.3)‡	38.2 (14.6, 193.6)	0.006
Day 14	72.4 (11.4, 98.2)§	45.3 (21, 133.8)	<0.0001

Data presented are median (interquartile range).
Comparison between groups with the Mann-Whitney U test.
Comparison within groups with the Friedman rank test and singular ranges with Wilcoxon using ranges test.
*Compared with baseline, $P < 0.05$.
†Compared with day 7, $P < 0.05$.
‡Compared with baseline, $P = 0.084$.
§Compared with baseline, $P = 0.07$.

Table 5
Multivariable logistic analyses for total parenteral nutrition–associated cholestasis

Variable	OR	95% CI		*P* value
		Lower	Upper	
Model 1				
Age at start of TPN (d)	0.483	0.216	1.078	0.076
TrophAmine	2.040	0.313	13.298	0.456
Model 2				
Age at start of TPN (d)	0.247	0.064	0.951	0.042
Methionine concentration (14 d)	1.076	0.994	1.165	0.069
Cysteine concentration (14 d)	0.985	0.966	1.005	0.134
Taurine concentration (14 d)	0.962	0.905	1.024	0.222

CI, confidence interval; OR, odds ratio; TPN, total parenteral nutrition.

the group of patients who received the TrophAmine solution. Children with cholestasis also had an increase in liver enzyme levels.

Discussion

To our knowledge, this is the first randomized clinical trial to analyze and compare the plasma concentrations of methionine, cysteine, and taurine and incidence of TPNAC in neonates on TPN receiving either an intravenous amino acid solution based on the breast milk aminogram or an intravenous solution of pediatric amino acids based on the umbilical cord aminogram. The analysis of the amino acid concentration at day 14 of TPN indicated that the plasma concentration of taurine was significantly higher and the concentration of plasma methionine was significantly lower in the Primene group than in the TrophAmine group.

Many studies have reported the close relationships among the development of cholestasis and lack of enteral intake, pathologic conditions such as sepsis and short-bowel syndrome, prematurity, and the duration and components of TPN [1,2]. In this study, in the group of infants who received an intravenous amino acid solution based on the breast milk aminogram, high concentrations of methionine and lower concentrations of taurine may be considered another risk factor, in addition to the inherent factors already mentioned. In neonates the capacity for synthesis is limited because of a decrease in the enzymatic activity of the hepatic transsulfuration pathways [27].

Although the cause of neonatal cholestasis is multifactorial, as we mentioned previously, there is evidence that administration of taurine may prevent cholestasis in the neonate [1,22,28], whereas high doses of some plasma amino acids such as methionine can lead to elevated concentrations of amino acids in the blood and cause toxicity as a result of the immaturity of degradation pathways and limited renal function for nitrogen excretion [1,3,29].

The mechanism proposed by which methionine induces the development of cholestasis may be related to altering the canalicular flow and permeability of the membrane, reducing biliary flow and producing an accumulation of hepatotoxic bile acids [9,29,30].

On the other hand, although taurine promotes biliary flow and protects against the toxicity of lithocholic acid, a proportion of our patients in the Primene group presented with cholestasis, even with higher taurine concentrations, in relation to those neonates who presented cholestasis in the TrophAmine group. This could be because the clinical conditions inherent to the patient can favor cholestasis.

A few studies have investigated and compared the effect of intravenous amino acid solution based on the breast milk aminogram versus an intravenous solution of pediatric amino acids based on the umbilical cord aminogram on plasma amino acids levels in neonates receiving TPN. One of these studies assessed the effect of these two parenteral solutions of amino acids on leucine turnover in preterm infants [31]. Another report of a retrospective study compared only the effect of these solutions of amino acids on TPNAC without measuring the plasma aminogram [32]. A study by Bulbul et al. [23] in very low birthweight infants who received parenteral nutrition starting with 1.0 $g \cdot kg^{-1} \cdot d^{-1}$ or 3.0 $g \cdot kg^{-1} \cdot d^{-1}$ of Primene solution found that the plasma levels of methionine did not increase during the administration of TPN, which is unlike our results and reports by other authors [1,3, 23,29]. A study by Coran [33] found that methionine levels are elevated in serum after just 1 wk of TPN in infants and that in those infants who died of TPNAC and cirrhosis, methionine levels were markedly elevated shortly before death [28]. In this work, there were significant increases in the concentrations of methionine in both groups between baseline and day 14 of TPN. However, in the infants assigned to the TrophAmine group, the plasma concentration increased by 110% versus 66% in the infants who received Primene. This is consistent with the finding reported by Poindexter et al. [20] in a group of infants receiving TrophAmine, in which there were significant increases in the concentration of methionine between baseline samples and those from approximately day 10 of TPN (28 μmol/L versus 40 μmol/L).

Regarding the incidence of TPNAC, in our study, only 3 out of 14 patients receiving Primene and 6 out of 21 receiving TrophAmine developed cholestasis. Unfortunately, this small number was not enough to generate significant statistical analysis. In Mexico, Carsi-Bocanegra et al. [34], in a retrospective study, reported a prevalence of cholestasis of 6.9% in preterm neonates. However, the amino acid solutions used for PN were not described. The incidence of cholestasis found in our infants receiving TrophAmine was greater than that reported by Wright et al. [15] (12.8% versus 28.6%) but lower than that reported by Robinson et al. [35] (58% versus 28.6%), whereas Aroor et al. [36], in a retrospective analysis of 91 low-birthweight infants who received the Primene amino acid solution for early (14.3 h after birth) and late (47.3 h after birth) PN, reported a 12.5% and 12.1% prevalence of cholestasis, respectively ($P = 0.96$), which are lower rates than that reported in our study (21%). Ozlü et al. [32], in a retrospective study, compared the effect of two different PN regimens on TPNAC using TrophAmine and Primene and reported a cholestasis rate of 27.9%, which was significantly higher in the high-dose group (receiving up to 3.5 $g \cdot kg^{-1} \cdot d^{-1}$ of amino acid) than in the low-dose group. Although this study is the only one to explore the effects of these two solutions on TPNAC, it presents some limitations, such as the design and use of the two solutions of amino acids in both groups of PN regimens, which does not allow an analysis of the differences in the frequency of cholestasis by type of amino acid solution administered [32].

One of the most important factors in the development of TPNAC is the beginning of enteral stimulation. In our study the Primene group started at day 7, whereas in the TrophAmine group, there were neonates who started the enteral stimulation, a protective factor for cholestasis, from the first day of TPN.

Adverse events are relatively common among all neonates irrespective of allocation within a trial [37]. This study, carried out in a vulnerable population, illustrates the practical difficulties in conducting a randomized controlled trial in neonates as a result of numerous unpredictable losses during the clinical trial.

We recognize some limitations in our study. For example, the final sample size was affected by the number of infants who did not complete the study, in part because a significant percentage of them presented a good tolerance for enteral feeding (32.2%). TPN had to be suspended in other infants because of adverse outcomes (e.g., central venous catheter infection, dysfunctional catheter, accidental extraction, or rupture of the central venous catheter) or the severity of disease, and 7.5% of the infants died from complications

independent of TPN. Unfortunately, we evaluated cholestasis only up until day 14, which is early progression of TPNAC and likely not enough time to follow-up.

In contrast, the principal strength was the design used because a randomized controlled clinical trial allowed us to evaluate variations in the concentrations of plasma amino acids during the first 14 days of TPN, based on a rigorous methodology. On the other hand, as we have already stated, the number of patients recruited at the time of this analysis was insufficient to identify differences in the incidence of cholestasis between the groups; however, our analysis on the changes in plasma amino acids found a consistent and significant difference between the plasma concentrations of methionine and taurine at day 14 between the groups. Based on the results of plasma aminograms, we might suggest that the use of Primene solution is a better option over TrophAmine in infants receiving TPN to improve their prognosis, particularly in those who require a longer course of TPN.

Conclusions

In conclusion, in this study the administration of an intravenous solution of pediatric amino acids based on the umbilical cord aminogram yielded a higher taurine plasma concentration and lower methionine plasma concentration than did the administration of a similar solution based on the breast milk aminogram, which could cause less liver damage and consequently a lower incidence of TPNAC development in the neonate.

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