A randomised controlled trial of glutamine-enriched neonatal parenteral nutrition in Malaysia

Mohamad Ikram I, Quah B S, Noraida R, Djokomuljanto S, Faris Irfan C Y, Van Rostenberghe H

ABSTRACT

Introduction: The addition of glutamine to parenteral nutrition (PN) in neonates has not shown significant benefits as compared to adults thus far. This study aimed to determine the potential benefits of the addition of glutamine to neonatal PN in a tertiary hospital in a middle-income country.

Methods: This was a double-blinded randomised controlled trial. Babies who were admitted to the neonatal intensive care unit (NICU) and who required PN were eligible for inclusion in the study. The subjects were randomised to receive either glutamine-added PN (intervention) or standard PN (control). The most important outcomes included time to full enteral nutrition, incidence of sepsis and necrotising enterocolitis (NEC), clinical or culture-proven sepsis.

Results: Out of 270 subjects, 132 were randomised to the intervention group and 138, to the control group. Baseline data were comparable in both groups. The median time taken to reach full enteral nutrition was similar for both intervention and control groups (six days in each group, p-value is 0.52). The incidences of NEC, clinical sepsis and culture-proven sepsis did not differ significantly in the intervention and control groups (5.8 vs. 7.1 percent, p-value is 0.68; 15.7 percent vs. 10.2 percent, p-value is 0.21 and 16.5 percent vs. 15.7 percent, p-value is 0.38, respectively). Other outcomes such as duration of ventilation, duration of NICU stay and a subgroup analysis for preterm and term babies also showed no statistically significant differences.

Conclusion: Addition of glutamine to neonatal PN was not shown to improve outcome.

Keywords: glutamine, neonate, parenteral nutrition, randomised controlled trial, sepsis

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INTRODUCTION

The addition of glutamine to parenteral nutrition (PN) has proven beneficial for critically ill adults.[1,2] It was seen to have reduced the incidence of infectious complication rates and facilitated a shorter hospital stay without any adverse effect on mortality.[3] Glutamine is an essential fuel for enterocytes and white blood cells and plays an important role in cell division, acid-base balance and the antioxidative process. Although glutamine can be synthesised by humans, it could become a conditionally essential amino acid when the need for it increases in critically ill patients. Neonates, especially preterms, would benefit more from glutamine addition, since reserves in these patients could be less and their synthesising capacity could be immature. Surprisingly, however, there has been no study that describes the similar benefits of glutamine in neonates[4] as those seen in adults.

Most of the published studies in neonates[5-7] have been carried out in high-income countries where the infection rate is low, nutrition and antenatal follow-up of expecting mothers is optimal and incidences of small- for-gestational-age babies and necrotising enterocolitis are relatively low compared to those in developing nations.[8] Hence, it is likely that the benefits of glutamine for neonates, if any, would be easier to demonstrate in the setting of a developing nation, where malnutrition is more common and infection rates are higher than in high-income countries. All previous studies have focused exclusively on preterm neonates.

The aim of this study was to determine the benefits of added glutamine to PN in Hospital Universiti Sains Malaysia (HUSM), which is situated in the North East of Peninsular Malaysia, a middle-income country. Since previous studies have failed to demonstrate glutamine benefits in preterm babies, term babies were included in this study; they may also more closely represent the adult population, in whom the benefits of glutamine have been quite conclusively demonstrated.

METHODS

The study was conducted at the neonatal intensive care unit (NICU) of HUSM, a tertiery centre in Kubang

Paediatric Department, Universiti Sains Malaysia, Kubang Kerian 16150, Malaysia

Mohamad Ikram I, MD
Paediatrician and Senior Lecturer

Quah BS, MD
Consultant and Professor

Noraida R, MD
Associate Professor

Djokomuljanto S, MD
Paediatrician

Faris Irfan CY, MSc
Science Officer

Van Rostenberghe H, MD
Consultant and Professor

Correspondence to:
Dr Mohamad Ikram Ilias
Tel: (60) 9767 6537
Fax: (60) 9765 3370
Email: drikram@kk.usm.my

Table I. Baseline data.

Variable	No. (%)		p-value
	Intervention	Control	
Mean birth weight ± SD (kg)	2.15 ± 0.91	2.22 ± 0.94	0.529
Type of gestation			
Term (n = 94)	45 (37.2)	49 (38.6)	0.821
Preterm (n = 154)	76 (62.8)	78 (61.4)	
Type of delivery			
SVD	68 (51.1)	65 (48.9)	0.428
LSCS	53 (46.1)	62 (53.9)	
Gender			
Male	60 (49.6)	65 (51.2)	0.802
Female	61 (50.4)	62 (48.8)	

SVD: spontaneous vaginal delivery; LSCS: lower segment caesarean section

Kerian, Malaysia from August 2006 to January 2008. All newborns aged < 72 hours, who were admitted to the NICU in HUSM and who required PN were eligible to be included in the study. Babies identified to have chromosomal abnormalities clinically, those suspected or confirmed to have an inborn error of metabolism, those born from a consanguineous marriage and those anticipated to require < 48 hours of PN were excluded from the study.

Babies who were randomised to the control group received the standard PN used in HUSM. The standard PN was prepared based on Vaminolact (Frasenius Kabi, Bad Homburg, Germany) and contained all the nutrients and electrolytes required by sick neonates. Patients randomised to the intervention group received the same PN solution with added glutamine in the form of Dipeptiven® (Frasenius Kabi, Bad Homburg, Germany), which is a 20% solution of the dipeptide N (2)-L-alanyl-L-glutamine (ala-gln). It is stable during heat sterilisation and storage, and is highly soluble in water (568 g/L). The Dipeptivan® dosage aimed for during the study was approximately 0.6 g/kg/day. The amount of glutamine received in the intervention group varied slightly with the incremental volumes given to newborn babies as per the hospital protocol. All babies were also commenced on intralipids 20% at Day 2 of birth. The PN solutions for both groups were prepared in the pharmacy and were physically indistinguishable by the naked eye. All the investigators and treating medical staff were blinded to the group allocation of each baby throughout the duration of the study. The primary and secondary outcomes were measured and the infants were monitored from the day of admission until the day of discharge from the NICU or death.

The primary outcome measure was the time taken to reach full enteral nutrition (FEN). Important secondary outcomes included incidence of necrotising enterocolitis, number of sepsis episodes (clinical sepsis or culture positivity), time to discharge from the NICU, time to achieve extubation and number of blood transfusions during admission to the NICU. Clinical sepsis for the purpose of the study was defined as presence of two or more of the following: (1) negative blood culture; (2) radiological changes of pneumonia; (3) clinical sepsis (apnoea, hypo/hyperglycaemia, lethargy, fever, feeding intolerance, metabolic acidosis); (4) laboratory parameters: white cells >25 × 10^9/L or <5.0 × 10^9 /L, thrombocytopenia platelet <100 × 10^9/L, positive C-reactive protein > 10 mg/L.

The number of subjects required was calculated based on the primary outcome measure (mean difference in time taken to reach FEN) with a power of 0.9 and a two-tailed alpha of 0.05. Estimation of differences was made based on a previous randomised controlled trial of parenteral glutamine in ill, very low-birth weight neonates.[5] For some of the important secondary outcome measures, the study did not have sufficient power to identify significant differences, but trends could be identified.

The subjects were randomised into two groups using the first (and original) generator, which randomises each subject to a single treatment by using the method of randomly permuted blocks (www.randomization.com). The randomisation sequence was prepared by a person who was not directly involved in recruitment or care of the babies. The hospital pharmacist was responsible for the preparation of the appropriate solution according to the randomisation sequence that was provided to her. Initially, 240 subjects were randomised; however, another 30 subjects were subsequently randomised to compensate for the number of babies requiring < 48 hours of PN, thus giving a total of 270 subjects. At the time of the decision to include babies in the study, the investigators were not aware of the allocation of the next subject, ensuring concealment of allocation. Investigators and the health staff involved in the care of the patients were blinded to the allocation throughout the data collection period. The study was carried out after obtaining approval and written parental consent by the research and ethics committee of University Sains Malaysia. Data were entered and analysed using the Statistical Package for the Social Sciences Version 12.0.1 (SPSS, Chicago, IL, USA). The time taken to reach FEN, time to achieve extubation and length of NICU stay were analysed using Kaplan-Meier plot.

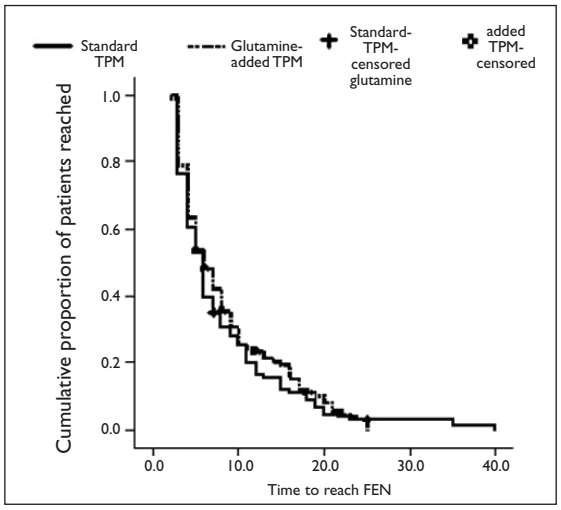

Fig. 1a Kaplan-Meier plot for time to full enteral nutrition.

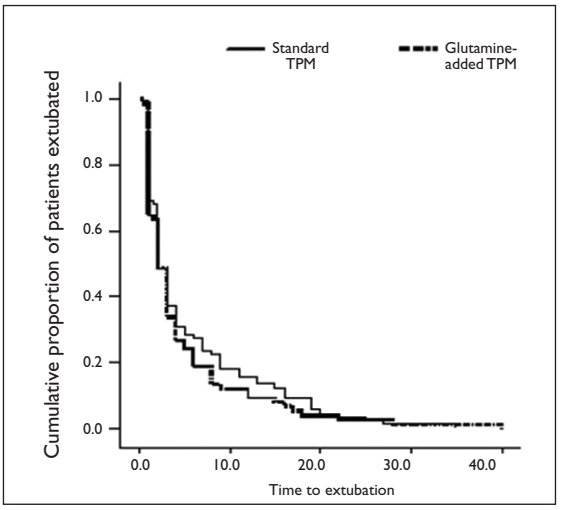

Fig. 1b Kaplan-Meier plot for time to extubation.

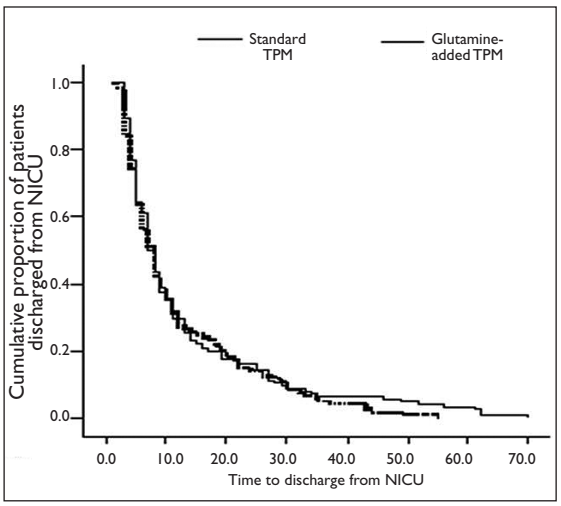

Fig. 1c Kaplan-Meier plot for time to discharge from NICU.

Incidence of sepsis, which included positive culture and clinical sepsis, was analysed using Pearson chi-square test. Subgroup analyses for both preterm and term babies were also performed.

RESULTS

A total of 270 babies were initially enrolled, with 132 babies in the intervention group and 138 in the control group. 11 babies from each group were excluded as they required < 48 hours of PN, thus leaving 121 babies in the intervention group and 127 babies in the control group, respectively, for analysis. The baseline data in both groups were not statistically different (Table I). 121 babies in the intervention group and 127 babies in the control group survived to reach FEN and were subsequently discharged from the hospital. From the Kaplan-Meier plot (Fig. 1a), the median time taken to reach FEN was six days (25th quartile: four days, 75th quartile: 11 days) in both intervention and control groups. Cox proportional hazards regression analysis indicated that the median time to reach FEN was the same for both the intervention and control groups (χ^2 change 0.35; p = 0.55; hazards ratio 1.08; 95% confidence interval [CI] 0.83–1.41).

A total of 162 babies required ventilation (74 in the intervention and 88 in the control group). The Kaplan-Meier plot in Fig. 1b shows that the median time to achieve extubation was two days each in the intervention (25th quartile: one day, 75th quartile: five days) and control (25th quartile: one day, 75th quartile: seven days) group. Cox proportional hazards regression analysis indicated that the median time to achieve extubation in both groups was the same (χ^2 change 0.33; p = 0.57; hazard ratio 0.91; 95% CI 0.67–1.25).

The Kaplan-Meier plot (Fig. 1c) demonstrated that the median time for discharge from the NICU was eight days (25th quartile: four days, 75th quartile: 15 days) in the intervention group and seven days (25th quartile: five days, 75th quartile: 14 days) in the control group, and this difference was also not significant (p = 0.47). Cox proportional hazards regression analysis indicated that the median time to discharge from NICU was one day shorter in the control group (χ^2 change 0.47; p = 0.49; hazard ratio 0.92; 95% CI 0.71–1.18).

The incidence of necrotising enterocolitis in both intervention and control groups was comparable and not statistically significant (p = 0.68). The total number of term and preterm babies included in the study is showed in Table II. All the outcomes were analysed separately and did not show any statistical difference in both groups. Table III shows the overall causes of death for babies included in the study. The majority of babies who died

Table II. Outcome data.

Variable	No. (%)		p-value
	Intervention	Control	
Mean duration of ventilation period ± SD (days)	4.82 ± 6.73	5.59 ± 6.94	0.480
Mean length of NICU stay ± SD (days)	11.96 ± 11.25	12.90 ± 13.94	0.560
Clinical sepsis	19 (16.0)	13 (10.5)	0.206
Total white blood cells (> 25 x10^9/L)	8 (6.8)	5 (4.0)	0.335
Platelets (< 100 x10^9/L)	14 (12.0)	14 (11.3)	0.870
NEC	7 (5.8)	9 (7.1)	0.677
Culture	20 (54.1)	20 (45.9)	0.383
Transfusion	51 (42.1)	60 (57.9)	0.420
CRP	34 (28.3)	25 (20.3)	0.146

NICU: neonatal intensive care unit; SD: standard deviation; NEC: necrotising enterocolitis; CRP: C-reactive protein

during the study were cases of confirmed septicaemia, as evidenced by positive blood culture. Four babies who suffered from complex cyanotic heart disease died due to the underlying condition; the babies were inoperable and received palliative care. Babies who died from severe respiratory distress syndrome had extremely low birth weights.

DISCUSSION

In this double-blind, randomised controlled trial, the effects of adding glutamine to standard total PN (TPN) for neonates were evaluated in the setting of a middle-income country. Previous studies in high-income countries had shown no benefits of adding glutamine to standard TPN solution for preterm babies.[4,5,7] However, in middle- and low-income countries, differences in maternal nutrition, antenatal care and postnatal infection rate may cause the benefits of adding glutamine to TPN for neonates to become apparent. Till now, there has only been one recent report of a study done in a middle-income country, which also failed to show benefits of glutamine for preterm babies.[9] The main difference between that study and the present study is that the sample size in the former study was much smaller than ours (53 vs. 270), and the study included only preterm babies. Also, more outcome measures were evaulated in our study.

Since glutamine is the major fuel for enterocytes and lymphocytes, and since conditions causing significant stress could render glutamine a conditional essential amino acid, in the setting of middle-income countries, glutamine was expected to have an effect on the time taken to FEN and the incidence of sepsis. However, this study showed that none of the outcomes differed significantly between the study group and the control group. These results are comparable with those of the studies done in the United States and China.[5,9] It

Table III. Causes of mortality in control and intervention groups.

Cause (n = 29)	No. of patients	
	Intervention (n = 14)	Control (n = 15)
Underlying disease		
Complex cyanotic heart disease	3	1
Meconium aspiration syndrome with PPHN	2	1
Severe asphyxia with HIE	2	1
Diaphragmatic hernia with lung hypoplasia	-	2
Severe RDS	1	2
Sepsis	6	8

PPHN: persistent pulmonary hypertension of the newborn; HIE: hypoxic ischaemic encephalopathy; RDS: respiratory distress syndrome

is still difficult to explain why addition of glutamine to TPN shows important and significant benefits for adults undergoing intensive care and not for preterm babies. One reason could be that glutamine is well transported through the placenta. Even preterm babies may either have built up reserves of or increased synthesising capacity for glutamine. An alternative explanation could be that enterocytes and leukocytes of neonates have a different metabolism and that glutamine is not as important as a fuel for these cells of the preterm neonate as it is for adults. It could also be that neonates require a higher dose of glutamine for effects similar to those in adults to become apparent. However, the dose of glutamine given in this study was comparable to that used in other studies in neonates and also to the dose/kg used in adults.

Glutamine is also an important precursor of glutathione. Glutathione plays an important role in the defence of red blood cells to oxidative stresses. Hence, the need for transfusion was also included as an outcome for this study. This was not a measured outcome in any

of the previously reported randomised controlled trials on the addition of glutamine in the PN. Although sick neonates are exposed to a large variety of oxidative stresses, the need for transfusion was not found to differ in babies receiving glutamine-enriched PN and those receiving standard PN.

When a subgroup analysis was performed for preterm babies, the primary outcome still did not differ between the study group and the control group. Also, most of the secondary outcomes showed no difference, except for time to extubation. The time taken to achieve extubation, reflecting the duration of mechanical ventilation, was shorter in the group receiving glutamine-enriched TPN and statistically almost significant ($p = 0.07$). This potential benefit was shown in only one previous study.[6] The subgroup analysis for term babies was particularly interesting, since preterm babies do not seem to benefit from adding glutamine to TPN. Adults do benefit, and term babies may be closer to adults in terms of physiology than preterm babies. In this study, the group of term babies was small ($n = 94$), and the study did not have enough power to draw firm conclusions regarding this subgroup. Although there were no significant differences, a trend was shown toward worse outcomes for the term babies, which was contrary to expectations.

In terms of side effects, no babies reported unexplained metabolic complications or increase in blood urea during the study. However, since it was adequately proven in previous studies that glutamine is safe for babies, blood urea and serum bicarbonate levels (which were routinely monitored in the NICU babies) were not systematically analysed. Plasma ammonia levels were also not systematically determined during the study. The strength of the study was that the study was a randomised controlled trial with double blinding, and was sufficiently powered to detect expected differences in the primary outcome. This was the first study that included term as well as preterm babies. It was also the first study to determine the need of transfusion in babies receiving glutamine-enriched TPN versus those receiving standard TPN. The limitations of this study include the lack of power to show small or even moderate differences in some of the important secondary outcomes and also in the subgroup analyses.

In conclusion, this study supports the existing literature that addition of glutamine to standard TPN does not confer additional benefits to preterm babies in the NICU. The small group of term babies included in this study showed a trend toward worse outcomes, which may indicate that a further trial focusing on term babies requiring TPN is not a priority research area.

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