

Original Research Article

A study of safety and effectiveness of amino-acids based multi-nutrient fortifier in preterm infants

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ABSTRACT

Background: Fortification plays a vital role in supporting the nutritional requirements in the premature infants. Currently in India, fortifiers are either whole protein based or whey protein which are bovine milk- based, which has been associated with increased risk of feed intolerance and necrotizing enterocolitis (NEC), to circumvent this, an amino-acids based fortifier has been made available which provides more proteins (0.35 g/1 g sachet) thereby meeting the nutritional requirements of preterm infants. Aim was to study effect (safety and efficacy) of fortification with the amino-acids based fortifier (HMF-ADVANCE, Analeptik Biologicals) in premature infants admitted in the neonatal intensive care unit (NICU).

Methods: A single centre pilot study was conducted on preterm infants with <1800 g birth weight and/or <32 gestational age, amino-acids based fortifier (AABF) was used as the fortification option once the infants reached 100 ml/kg/day feed volume. 1 g of HMF-ADVANCE was mixed in 25 ml of mother's own milk (MOM) and the fortification was continued till discharge from NICU.

Results: A total 100 preterm infants were included for the study, mean gestational age (GA) was 29.38±1.61 weeks, mean birth weight was 1159.90±301.9. Growth outcomes at the end of the study: mean weight gain was 23.73±7.62 g/day, mean head circumference gain was 1.02±0.41 cm/week, mean length gain was 1.01±0.15 cm/week and weight gain velocity were 17.13±2.38 g/kg/day. Blood urea nitrogen (BUN) showed an increasing trend from baseline (before starting fortification: 3.2 mg/dl), 1 week after fortification (6.3 mg/dl) and at the end of study (11.2 mg/dl). AABF was also used in three neonates post NEC. There were no episodes of feed intolerance or necrotising enterocolitis episodes and none of neonates discontinued fortification till discharge from NICU.

Conclusions: AABF was well tolerated without any feed intolerance episodes and helped to achieve optimal growth. AABF can be the optimal choice of fortification in low-birth-weight neonates.

Keywords: AABF, Preterm, Fortification, Amino acids

INTRODUCTION

Very-low-birth-weight (VLBW) neonates with intrauterine growth restriction (IUGR) often may have a severely impaired intestinal function.^{1,2} The preterm gut is further affected by the adverse blood redistribution that occurs in fetus with an IUGR. After birth, these infants

commonly respond to enteral feedings with abdominal distension, increasing volumes of gastric residuals, vomiting and necrotizing enterocolitis (NEC). The longer they are kept on total parenteral nutrition, higher would be the risk of catheter-related infections.^{3,4} Elemental feeds consisting of free amino acids would be more advantageous due its anti-inflammatory properties established in certain gastrointestinal ailments.⁵⁻⁸

Protein is an essential nutrient that plays a crucial role in supporting growth and development, immune function, and tissue repair. Preterm infants are particularly in need of protein due to their reduced muscle mass and strength compared to term infants. As a result, the optimal protein source and absorption rate is of great importance.⁹

Preterm infants have lesser gastric protein digestion capacity than term infants. The research suggests that amino acids are better absorbed from the stomach compared to intact proteins due to their smaller size, direct absorption into the bloodstream, and independence from digestive enzyme activity and intestinal transit time. This highlights the importance of considering the absorption rate and efficiency of protein sources when selecting a protein source for preterm infants.^{10,11}

Meta-analysis by Wang et al concluded that oral amino acids supplementation particularly oral arginine may reduce severe NEC, and oral glutamine may reduce LOS and the time to reach full feeding in preterm infants.¹²

ESPGHAN 2023 has provided the following statement on benefits of amino acids “In addition to being important for growth, individual amino acids may have selective functions. These include glutamine (immune function), arginine (gut health), and taurine (brain development)”.¹³

Preterm infants require greater nutritional support than term infants for adequate growth and development. Many research studies have shown that feeding of unfortified human milk resulted in inadequate weight gain and growth.

Consequently, fortification of the human milk is very much essential to meet the demands of macronutrients in very low and extremely low birth weight preterm infants. Bovine-milk-based fortifiers are the commonly used fortifiers in India, which contain intact proteins.¹⁴

Feeding bovine milk derived proteins may adversely affect gut condition, induce gut epithelial cell damage, and ultimately lead to dysbiosis, especially in premature infants.^{15,16}

Amino acids-based formula has been used in preterm infants since many years and has shown benefits such as better growth, improved feed tolerance in premature infants when compared with intact proteins-based formula in published studies.¹⁷

Recently the amino-acids based fortifiers (AABF) are made available in India, availability of AABF would provide additional benefits when compared with the conventional intact proteins-based fortifiers such better protein bio-availability, adequate growth and lesser feeding intolerance, to understand the benefits of AABF this exploratory study was initiated to assess the effects on feed tolerance, growth and other benefits of AABF.

METHODS

Study design

This was an observational exploratory study conducted at Motherhood Hospital, Indiranagar, Bangalore between April 2023 to December 2023. The study was initiated after receiving the approval from the independent ethics committee and informed consent was obtained from the parents prior to the commencement of the study.

Inclusion criteria

Neonates with birth weight <1800 g and/or <32 weeks gestational age (GA) were included for study.

Exclusion criteria

Neonates who received any bovine milk-based formula/fortifier prior to this study and neonates diagnosed with major congenital malformations or intestinal anomaly were excluded from the study.

Study procedure

All infants received intravenous nutrition and trophic feeds was initiated at the earliest with mother's own milk or pasteurized donor human milk when mother's own milk was not available or insufficient. Feeds with mother's own milk or donor human milk was increased with an increment of 10-15 ml/kg/day, as per the hospital neonatal intensive care unit (NICU) protocol. Fortification of human milk was initiated when the enteral feed volume reached more than 100 ml/kg/day. Infants received expressed breast milk (EBM) fortified with HMF-ADVANCE (amino-acids based fortifier, Analeptik Biologicals, n=100). Double dilution protocol was used for the initial 3 days of fortification, where 1 g HMF-ADVANCE was mixed in 50 ml of EBM for the first 3 days, post which standard fortification protocol was followed, where 1 g HMF-ADVANCE was mixed in 25 ml of EBM. Feeds were advanced as per the tolerance of infants to enteral feeding and fortification with AABF was continued till discharge from NICU. Osmolality of AABF was noted to be 380 mOsm.

Data was recorded in the case record form (CRF), data parameters included birth weight, gestational age at birth, occipital frontal circumference (OFC) and length at birth, APGAR scores at one and 5 minutes after birth, day of life (DOL) of starting enteral feeds, number of days on total parenteral nutrition, DOL of starting fortification, mean weight gain per day (g/d), feeding intolerance episodes, sepsis/infections (duration in days), diagnosis of NEC with grade (Bell's staging) , if any, other complications of prematurity. Other parameters included were number of days of antibiotics usage, biochemical tests including blood urea nitrogen (BUN), number of days of NICU stay, discharge weight, length, and OFC.

A total of 100 infants who satisfied the inclusion criteria were included for the study.

Outcome measures

Primary outcomes included growth during the fortification period, average weight gain (g/day), growth velocity (g/kg/d), length gain (cm/week), OFC gain (cm/week). Secondary outcomes included number of infants experiencing feeding intolerance events during fortification, incidence of infections/sepsis with requirement of antibiotics (days), length of hospital stay (days), incidence of NEC ($\geq$ Bell's stage II), biochemical markers for protein utilization: BUN, plasma calcium and phosphate levels.

RESULTS

Demographics

A total of 100 infants were included in the study, baseline characteristics are provided in Table 1.

Table 1: Demographics and baseline characteristics.

Parameter	AABF (n=100) (mean $\pm$ SD)
Female, n	52
Gestational age (weeks)	29.38 $\pm$ 1.61
Gestational age (weeks), median (min-max)	30.25 (25.5–32)
Birth weight (g)	1159.90 $\pm$ 301.9
Birth weight (g), median (min-max)	1210 (800–1480)
Antenatal steroid, n	72
Received MgSO ₄ , n	20
LSCS, n (%)	100
APGAR score at 1 minute	6.5 $\pm$ 0.93
Temperature at time of NICU admission (°C)	35.63 $\pm$ 1.09
Type of feeds	
MOM only, n	24
MOM+DHM, n	76
Need for respiratory support, n	100
Number of days of oxygen support	9.18 $\pm$ 5.62
TPN (days)	9.36 $\pm$ 3.9
Feed volume at initiation of AABF (ml/kg/day)	120 $\pm$ 12.6
Dol starting AABF (days)	11.2 $\pm$ 3.8
Weight (g) at end of study	1610.9 $\pm$ 19.1
Number of days on AABF	19.1 $\pm$ 8.08
Sepsis (culture positive), n	3

Primary outcome: growth during the fortification period, average weight gain (g/day), growth velocity (g/kg/d), length gain (cm/week), OFC gain (cm/week) (Table 2).

Table 2: Primary outcome–growth outcome measures.

Parameter	AABF (n=100) (mean $\pm$ SD)
Weight gain/day (g)	23.73 $\pm$ 7.62
Weight gain/day (g), median (min-max)	20.39 (16.32–28.35)
Head circumference gain/week (cm)	1.02 $\pm$ 0.41
Length gain/week (cm)	1.01 $\pm$ 0.15
Growth velocity (g/kg/day)	17.13 $\pm$ 2.38

Secondary outcomes: number of infants experiencing feeding interruptions after starting fortification, incidence of infections/sepsis with requirement of antibiotics (days), length of hospital stay (days), incidence of NEC ($\geq$ Bell's stage II), biochemical markers for protein utilization: BUN, plasma calcium and phosphate levels (Table 3).

There were no episodes of feed intolerance or NEC episodes during the study period, none of neonates discontinued fortification till discharge from NICU.

Table 3: Secondary outcome measures.

Parameter	AABF (n=100) (mean $\pm$ SD)
Feed intolerance, n (%)	0 (0)
NEC, n (%)	0 (0)
Length of hospital stay (days)	26.7 $\pm$ 10.2
BUN - baseline (mg/dl)	3.2 $\pm$ 2.21
BUN - after 1 week of fortification	6.3 $\pm$ 1.4
BUN - end of fortification (mg/dl)	11.2 $\pm$ 2.3
Mean plasma calcium (mg/dl)	9.45 $\pm$ 1.8
Mean plasma phosphate (mg/dl)	5.1 $\pm$ 1.6

Table 4: Subjects with history of feed intolerance or complications before initiation of AABF.

Parameter	AABF (n=100)
Feed intolerance, n	8
NEC, n	3

DISCUSSION

Increasing survival and improving growth of the preterm infant to avoid extrauterine growth restriction have resulted in demands for protein that present powdered fortifiers may not achieve. Although some of these infants may compensate with higher volume intake, many are unable to consume a sufficient volume because of pulmonary or other clinical issues and therefore require further concentration of protein and energy. Higher intake of protein between 3 and 4 g/kg/day has been associated with improved growth without complications compared with a lower consumption of protein (<3 g/kg/day).⁷ Poor

weight gain has been associated with a higher risk for retinopathy of prematurity and poor neurodevelopmental outcomes.¹⁸⁻²⁰

Study by Demers-Mathieu et al revealed that preterm infants have reduced gastric digestive capacity than term infants, this reduced digestive capacity can limit the preterm gut's ability to disintegrate the intact proteins and absorb amino acids, therefore restraining growth and development. Results of the study described that overall gastric protein digestion including proteolysis activity and general protease activity were significantly lower in preterm infants by 92.6% and 294% than term infants. It was noted that at 2-hour post-feeding preterm infants had lower gastric protein digestion capacity than term infants. Which implies that intact proteins such as whey proteins would require longer time for digestion and absorption in the gastro-intestinal tract, and only about 50% of orally provided intact proteins would be digested and absorbed from the preterm gut which would not meet the protein requirements of the premature neonates.¹⁰

A study by Weijzen et al reported that free amino acids showed better bioavailability of 76% than with intact milk proteins 59% provided by oral route, authors concluded that bolus ingestion of free amino acids leads to rapid amino acid absorption resulting in superior postprandial plasma amino acid bioavailability than ingestion of an equivalent amount of intact milk protein. Free amino acids may be preferred over intact proteins in conditions such as immature gut, post-surgical situations where protein digestion and absorption are compromised.¹¹

Meta-analysis by Wang et al concluded that oral amino acids supplementation has shown various advantages to preterm infants, where oral supplementation of amino acid arginine resulted in reduction of necrotizing enterocolitis (NEC) stage $\geq$ II (OR 0.48; 95% CI 0.26-0.90). Oral supplementation of amino acid glutamine reduced the likelihood of developing late onset sepsis (LOS) compared to placebo (OR 0.62; 95% CI 0.47-0.82); and can also reduce the time to reach full enteral feeding (MD= -2.63 days; 95% CI -4.99 to -0.27). Oral arginine may reduce severe NEC, and oral glutamine may reduce LOS and the time to reach full feeding.¹²

Kim et al compared the hydrolysed proteins-based fortifier with an intact protein-based fortifier in 147 preterm infants. Authors concluded that the weight gain in completely hydrolysed proteins-based fortifier was 18.2 g/kg/day and in intact protein-based fortifier was 17.5 g/kg/day. The hydrolysed proteins-based fortifier group achieved greater linear growth over time compared to the intact protein-based fortifier ($p=0.029$). Completely hydrolysed proteins were well tolerated with optimal weight gain.²¹

Tewari et al reported that mean weight gains with a commonly used bovine milk-based fortifier with intact proteins was found to be 9.2 ± 2.2 g/day, mean OFC gain

was 0.7 ± 0.2 cm/week and mean length gain was 0.8 ± 0.3 cm/week.¹⁴

Mukhopadhyay et al reported in their study that fortification with a bovine milk-based fortifier with intact proteins resulted in a mean weight gain of 15.1 ± 4 g/kg/day, mean OFC gain was 0.83 ± 0.2 cm/week, mean length gain was 0.86 ± 0.2 cm/week and 20.7% of the neonates experienced feeding intolerance.²²

In our study the mean weight gain was 23.73 ± 7.62 g/day, mean OFC gain was 1.02 ± 0.41 cm/week and mean length gain was 1.01 ± 0.15 cm/week. None of the infants experienced feeding intolerance episodes after initiation of AABF. These results suggest that the better weight gain and reduced feeding intolerance could be mainly due to better absorption and bio-availability of amino acids with AABF.

The biochemical markers of protein utilization, Blood urea nitrogen (BUN) showed an increasing trend from baseline (before starting fortification: 3.2 mg/dl), 1 week after fortification (6.3 mg/dl) and at the end of study (11.2 mg/dl), while the normal reference range for BUN in newborns would be between 9-14 mg/dl, which indicates the optimal protein utilization with AABF.

Three infants received fortification with AABF post NEC, the infants had no feeding intolerance episodes after the initiation of AABF, also 8 infants had history of feeding intolerance before fortification, who did not develop any feeding issues later on with initiation of AABF, the above provides preliminary evidence regarding the safety of AABF.

Major difference between the currently available fortifiers is mentioned in Table 5.

Table 5: Key difference between the currently available fortifiers.

S. no	Parameters	Bovine milk-based fortifier (1 g)	Amino acids-based fortifier (1 g)
1	Protein	0.27 g	0.35 g
2	Protein - type	Whole whey protein	Amino acids based
3	Bioavailability	Low	High
5	Feed intolerance (risk)	Moderate	Least
6	Allergic potential (risk)	High	Least

This was an exploratory observational study with interim analysis of 100 subjects, the study would be continued to reach a sample size of 500 subjects; hence findings of

significant difference can be considered as associations between the outcomes only. Based on the results from this study, larger prospective controlled randomized study on AABF should be done to assess the beneficial effects in premature infants.

Limitations

This was a single arm exploratory and observational study, although had a sample size of 100, further randomized controlled studies can be conducted, which would provide the extent of benefits of fortification with AABF.

CONCLUSION

This study provides the initial evidence with 100 subjects to support the usage of AABF in preterm babies with adequate growth. AABF was well tolerated without adverse effects, AABF can be optimal choice of fortification in low-birth-weight neonates.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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