Original Article

Effects of Amino-acids based fortifier in preterm infants – An open label, observational study

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ABSTRACT

Background: Preterm infants have elevated protein needs for optimal growth and development; However, current bovine milk-based fortifiers are associated with feed intolerance and necrotising enterocolitis (NEC). An amino acid-based fortifier (AABF) can enhance protein absorption and tolerance in preterm infants. This study evaluates the safety and efficacy of an amino-acid-based fortifier in improving growth outcomes in preterm neonates. **Methods:** Preterm infants with a birth weight < 2000 g and/or gestational age < 32 weeks were prospectively observed in this single-centre, open-label study. Following the establishment of enteral feeds at 100 ml/kg/day, an amino acids-based forifier (Human Milk Fortifier (HMF) -ADVANCE) was introduced and maintained until neonatal intensive care unit (NICU) discharge. The primary outcome was assessment of daily weight gain, with secondary outcomes including weekly gains in length and head circumference and the occurrence of feed intolerance was also considered for assessment. **Results:** A total of 25 preterm infants (12 males and 13 females) with an average birth weight of 1204.62 ± 48.9 grams and a mean gestational age of 31.33 ± 3.4 weeks were enrolled in the study. At the end of the study period, the observed growth outcomes included a mean weight gain of 21.34 ± 3.41 grams per day, a mean head circumference increase of 0.92 ± 0.32 cm per week, and a mean length increase of 0.9 ± 0.13 cm per week. There were no instances of feed intolerance or NEC during the study, and none of the infants required discontinuation of fortification before discharge from the NICU. **Conclusion:** Fortification with an amino-acid-based fortifier in preterm infants demonstrated positive growth outcomes with optimal weight gain, length gain, and head circumference growth. No infants exhibited feeding intolerance, indicating good safety and tolerance. These findings suggest that AABF is an effective fortification strategy for preterm neonates.

Key words: Preterm infants, Human Milk, Amino Acids, Weight Gain, Neonates, Nutritional Status

Preterm infants, particularly very low birth weight (VLBW) and extremely low birth weight (ELBW) infants, require increased nutritional support for optimal growth and development. Studies indicate that unfortified human milk feeding provides inadequate weight gain and suboptimal growth, thereby necessitating macronutrient supplementation [1]. In India, the most widespread approach to human milk fortification involves the use of bovine milk-based fortifiers [2].

Despite the widespread use of bovine milk-derived fortifiers for preterm infants' high protein needs, this practice may negatively impact their gut health. Research suggests these proteins can damage the gut epithelium, disrupt the microbiota, and lead to dysbiosis, particularly in this vulnerable population [3]. In contrast, the developing foetus receives a substantial amino acid supply (3-4 g/kg/day) crucial for growth and brain development [1], emphasising the importance of adequate protein intake, as deficiency can result in a negative nitrogen balance.

A comparison of preterm human milk composition with the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) recommendations reveals that exclusive breastfeeding fails to meet the nutritional demands of preterm infants [1, 4]. Consequently, the standard practice in most neonatal units is to fortify expressed breast milk (EBM) using commercially available human milk fortifiers (HMF) to bridge this nutritional gap. Growth, development, immune competence, and tissue repair in infants heavily rely on adequate protein intake. Preterm infants, characterized by reduced muscle mass and strength at birth compared to term infants, exhibit a increased demand for protein. Therefore, careful consideration of the protein source and its bioavailability is a critical aspect of their nutritional management.

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Amino acids and whey proteins are common protein sources for preterm infants, but amino acids are absorbed more efficiently. Free amino acids bypass the breakdown required for whey proteins, allowing for direct absorption into the bloodstream from the small intestine, leading to faster and more efficient uptake [5].

Factors Influencing Protein Absorption:

- Molecular Size: Compared to whey proteins that require enzymatic breakdown before absorption, free amino acids, being smaller in size, are readily absorbed in the small intestine.
- Intestinal Transit Time: Breakdown and absorption of whey proteins are disturbed due to a longer transit time. However, the absorption of amino acids remains unaffected.
- Digestive Enzyme Activity: Whey protein breakdown and absorption are impaired due to reduced activity of digestive enzymes. However, amino acids are absorbed efficiently regardless of enzyme activity [6, 7].

Given these factors, amino acids offer superior absorption efficiency compared to whey proteins, highlighting their importance when selecting an appropriate protein supplement for preterm infants. AABF have recently become available in India, and a study by Chandra P et al. has been published supporting their use [8]. The findings favour the use of AABF, and this study will contribute additional evidence on feed tolerance and growth in preterm infants receiving AABF.

MATERIALS AND METHODS

Before the commencement of the study, approval was obtained from an independent ethics committee, and written informed consent was obtained from the parents of all participants. All infants received early trophic feeds with the mother's milk or pasteurised donor human milk when needed. Following the hospital Neonatal Intensive Care Unit (NICU) protocol, human milk fortification started once enteral feed volume was greater than 100 ml/kg/day. This study included preterm infants with a birth weight under 2000 grams and/or a gestational age below 32 weeks. Infants with major congenital anomalies, significant feeding protocol deviations, or discharge against medical advice were not included in the study.

Infants were fed expressed breast milk fortified with Amino Acid-Based Fortifier (HMF-ADVANCE, Analeptik Biologicals), which was mixed with 25 mL of expressed breast milk (EBM). Feed advancement was guided by the infant's tolerance to enteral feeding. All data were documented in the case record form (CRF).

A total of 29 infants were screened for the study, of which 25 satisfied the inclusion criteria. All infants received care according to the hospital's NICU protocols and guidelines. This included intravenous nutrition during the initial hours after birth, when clinically indicated. Enteral feeding was initiated at the earliest opportunity using expressed mother's milk (MOM), or pasteurised donor human milk when MOM was unavailable. Enteral feedings were advanced gradually based on a predefined protocol, taking into consideration the infant's gestational age and birth weight. Once enteral intake exceeded 100 ml/kg/day, fortification of human milk with AABF was initiated for all infants. Fortification was maintained until discharge from the NICU.

Outcomes and Statistical Analysis

Primary outcomes included growth and average weight gain (g/day). Secondary outcomes included length gain/week (cm/week), head circumference gain/week (cm) and incidence of feed intolerance. Descriptive statistics expressed in terms of mean and standard deviation, and distribution or frequencies were expressed as percentage.

RESULTS

The study enrolled 25 preterm infants, with a roughly balanced sex ratio, showing a minor overrepresentation of female infants. All deliveries were performed via Lower Segment Cesarean Section (LSCS). A significant proportion of the infants (72%) received antenatal steroids, and one-fifth (25%) were administered magnesium sulfate. A post-AABF administration increase in birth weight of approximately 500 grams was observed. Table 1 provides a comprehensive overview of the demographic and baseline characteristics of the study participants.

Table 1: Demographics and baseline characteristics

Parameter	Mean ± Standard Deviation
Gestational age (weeks)	31.33 ± 3.4
Birth weight (g)	1204.62 ± 48.9
Weight (g) at end of study	1676.11 ± 19.1
Feed volume at initiation of AABF (ml/kg/day)	140.2 ± 12.6
Dol starting AABF (days)	11.2 ± 3.8
Number of days on AABF	17.4 ± 4.2

Parameter	n (%)
Males	12 (48)
Females	13 (52)
LSCS	25 (100)
Antenatal steroid	18 (72)
Received $MgSO_4$	5 (20)

LSCS: Lower Segment Cesarean Section; AABF: amino acid-based fortifier; $MgSO_4$: Magnesium sulfate.

Table 2 summarizes the primary outcomes, including average weight, and gains in length and head circumference. The average weight gain per day was approximately 21 grams, while the mean increases in head circumference and length reached up to 0.9 cm. Regarding secondary outcomes (Table 3), no significant adverse effects were observed, as evidenced by the number of infants without feeding interruptions after starting fortification and the absence of necrotizing enterocolitis (≥ Bell's stage II)

Table 2: Primary and secondary outcomes – growth outcome measures.

Parameter	Mean ± Standard Deviation
Weight gain/day (g)	21.34 ± 3.41
Head circumference gain/week (cm)	0.92 ± 0.32
Length gain/week (cm)	0.9 ± 0.13

Table 3: Secondary outcome measures: safety outcome measures.

Parameter	n (%)
Feed intolerance	0 (0%)
Necrotising Enterocolitis (NEC)	0 (0%)

DISCUSSION

Research suggests a potential association between widely used bovine milk-based fortifiers for preterm infants and an increased risk of feeding intolerance [3], raising questions about their overall impact on gut health, despite their nutritional benefits. To achieve optimal postnatal growth and prevent extrauterine growth restriction in a population with increasing survival, preterm infants' protein requirements frequently outstrip the provision from existing powdered human milk fortifiers. Since many cannot tolerate the higher feeding volumes needed for compensation due to pulmonary or other clinical issues, a concentrated supply of protein and energy becomes a clinical necessity [9]. This aligns with research demonstrating that higher protein intake (3-4 g/kg/day) improves growth outcomes without increased complications, unlike lower intake (<3 g/kg/day) [1].

Poor weight gain in preterm infants correlated with increased risk of retinopathy of prematurity and adverse neurodevelopmental outcomes. Demers-Mathieu et al. showed that preterm infants have lower gastric digestive capacity than term infants, limiting their ability to digest milk proteins and absorb amino acids. Despite active milk proteases in the infant stomach, their overall contribution is minimal; their optimal activity at near-neutral pH suggests a greater role in the small intestine. Gastric pepsin activity and protein digestion are also reduced in early postnatal preterm infants [7].

Weijzen et al. demonstrated that ingestion of free amino acids results in more rapid absorption and higher postprandial plasma amino acid levels compared to equivalent amounts of intact milk protein [10]. This finding suggests that free amino acid supplementation may be advantageous in clinical conditions where protein digestion and amino acid absorption are compromised [10].

In a comparative study, Kim et al., evaluated a hydrolyzed amino acid-based fortifier against an intact protein-based fortifier in 147 preterm infants. Their finding revealed that the group receiving the amino acid-based fortifier demonstrated significantly greater weight gain (18.2 g/kg/day vs. 17.5 g/kg/day) and superior linear growth over time (P=0.029). The study concluded that the hydrolyzed amino acid-based fortifier was well tolerated and effectively supported optimal growth [11].

Chandra et al. studied AABF (HMF-ADVANCE) in 100 preterm infants, noting favourable growth outcomes: 23.73 g/day average weight gain, 1.02 cm/week head circumference gain, 1.01 cm/week length gain, 17.13 g/kg/day, weight gain velocity. Blood urea nitrogen (BUN) levels increased from 3.2 to 11.2 mg/dL, suggesting improved protein metabolism. AABF was well tolerated, including in three post-NEC cases, with no feed intolerance or NEC episodes, and no discontinuation until NICU discharge [8]. Consistent with prior research on amino acid-based fortifiers (AABF), no infants in our study experienced feeding intolerance after starting HMF-ADVANCE. These findings suggest that AABF can be incorporated into the nutritional regimen of preterm infants without evidence of associated adverse effects.

CONCLUSION

In preterm infants, HMF-ADVANCE proved effective in supporting optimal growth across weight, length, and head circumference metrics. Concurrently, its administration was associated with no observed feeding intolerance, highlighting both its efficacy and favorable safety profile. This strongly suggests HMF-ADVANCE as a viable and effective fortification strategy for preterm neonates.

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