www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2022;11(1):e110104
doi: 10.7363/110104
Received: 2020 Oct 07; revised: 2020 Dec 02; rerevised: 2021 Jan 14; accepted: 2021 Jan 19; published online: 2021 Nov 17

Original article

Effect of human milk enriched with human milk-based fortifier (HMBF) versus bovine milk-based fortifier (BMBF) on growth and morbidity among very low birth weight (VLBW) infants – A randomized controlled trial

Rakesh Kotha[1], Kalyan Chakravarthy Konda[2], Paramesh Pandala[1], Alimelu Maddireddi[1]

[1]Department of Neonatology, Niloufer Hospital, Hyderabad, Telangana, India
[2]Niloufer Hospital, Hyderabad, Telangana, India

Abstract

Introduction: Survival of preterm neonates increased dramatically with the advancement in neonatology services. These infants have high nutritional demands for optimal growth, and human milk alone is insufficient to meet their needs. Hence, fortification of milk has become the standard of care. Bovine milk-based fortifiers (BMBF) are commonly used, but there exists concern regarding the exposure to cow milk protein. Human milk-based fortifier (HMBF) use offers a theoretical advantage from the immunological and gastrointestinal standpoint. We intend to study the effects of HMBF compared to BMBF on growth and morbidity of preterm neonates.

Methodology: It is a single-centre, open-labelled, randomized controlled trial (RCT) enrolling very low birth weight (VLBW) neonates of less than 34 weeks of gestation weighing between 1,000 and 1,500 g. Only human milk was used, and infants were randomized to receive either fortifier after reaching 100 ml/kg/day of enteral feeds. Growth and morbidity of preterm neonates were analysed.

Results: A total of 50 infants were enrolled (25 in each arm). Weight gain (21.42 vs. 20.84 g/day, p = 0.77) and growth velocity (16.45 vs. 15.85 g/kg/day, p = 0.57) were similar in both groups with no statistical difference. Sepsis (relative risk [RR] = 0.6), feed intolerance (RR = 0.57), necrotizing enterocolitis (NEC) (RR = 0.33) and duration of hospital stay (33 vs. 36 days) were better in the HMBF group than in the BMBF group.

Conclusion: Growth velocity was similar in both groups. However, HMBF was well tolerated by neonates with lesser incidence of feed intolerance, NEC, sepsis, and lesser hospital stay duration than in neonates supplemented with

BMBF. Given the fewer number of studies, there is a need for well-powered RCTs with a good sample size to fill the knowledge gap.

Keywords

Human milk, BMBF, HMBF, VLBW, neonates, perinatology, nutrition.

Corresponding author

Dr. Rakesh Kotha, MD, DM, Assistant Professor of Neonatology, Niloufer Hospital, Hyderabad, Telangana, India; phone: 7780109243; email: dr.rakeshkotha@gmail.com.

How to cite

Kotha R, Konda KC, Pandala P, Maddireddi A. Effect of human milk enriched with human milk-based fortifier (HMBF) versus bovine milk-based fortifier (BMBF) on growth and morbidity among very low birth weight (VLBW) infants – A randomized controlled trial. J Pediatr Neonat Individual Med. 2022;11(1):e110104. doi: 10.7363/110104.

Introduction

Preterm infants account for 13.6% of total deliveries in India [1]. Their survival has increased dramatically with the advancement in neonatology services. During the intrauterine period, optimal growth rate occurs predominantly in the late 2nd trimester and 3rd trimester. Therefore, those infants delivered preterm have high nutritional demand for optimal growth rate. Mother's milk is an important source of nutrition for preterm and hospitalized infants in a Neonatal Intensive Care Unit (NICU) [2]. In the setting of inadequacy of mother's own milk (MOM), pasteurized donor human milk (PDHM) is a preferable alternative over formula owing to its better tolerability and lesser gastrointestinal side-effects [3, 4]. Though human milk has innumerable benefits (nutritional and non-nutritional) due to various bioactive components (vitamins, minerals, oligosaccharides, growth factors, immunoglobulins, cytokines, etc.), it alone does not provide sufficient nutrition to meet the high requirements of very low birth weight (VLBW) infants [5-7]. In order to meet the high energy and essential nutrient (such as proteins and minerals) requirements of the growing preterm neonates, fortification of human milk with macro- and micronutrients has come into practice [8-10].

Three procedures of fortification are known: standard, adjustable, targeted. "Adjustable" and "targeted" are two methods of individualized fortification. Both are advisable depending on the NICU experience and facilities.

"Standard fortification" refers to the addition of a fixed amount of fortifier to a fixed amount of feed volume. It is the most widely practiced method due to its ease and practical approach, but many infants continue to have suboptimal growth in spite of fortification. "Adjustable fortification" refers to adjusting the fortifier based on the metabolic response of the neonate. It is a better approach than the standard fortification, but requires frequent monitoring of blood urea nitrogen (BUN). "Targeted fortification" refers to fortifying after analyzing the macronutrient composition of the human milk. It is the ideal approach, but it is not practical, and it is labor-intensive. The European Milk Bank Association (EMBA) encourages the use of individualized fortification to optimize nutrient intake. Fortifiers used could be multi-nutrient fortifiers, which contain varying amounts of nutrients like protein, fat, vitamins and minerals, or single nutrient supplements, which contain only proteins, carbohydrates or lipids. Fortifiers could be derived from bovine milk or exclusive human milk [11-13].

Most of the available literature involves the use of bovine milk-based fortifiers (BMBF) for the fortification of human milk. In recent years, the use of a human milk fortifier derived from donkey milk has been investigated, starting from its biochemical similarity to human milk. It has been observed that it reduces the gastroesophageal reflux (GER) frequency, compared with BMBF in VLBW infants, and consequently is recommended in infants with feeding intolerance [14-16]. Though studies have shown improvement in short-term weight gain and early discharge with human milk fortified with BMBF over preterm formula, concern still exists regarding the exposure to cow milk protein and its associated adverse effects [17-18]. With the availability of human milk-based fortifier (HMBF), the use of exclusive human milk feeding offers a theoretical advantage from the immunological and gastrointestinal standpoint. There is also evidence supporting a lower incidence of retinopathy of prematurity (ROP) and sepsis apart from necrotizing enterocolitis (NEC) reduction with HMBF over BMBF [19]. There is a paucity of literature on the usage of HMBF for fortification, with only a few randomized controlled trials (RCTs) available [20]. Given the importance of exclusive human milk diet in the vulnerable VLBW infants versus the high cost of HMBF over BMBF, we intend to study the effects

of HMBF compared to BMBF on the morbidity of preterm neonates.

Methods and materials

This study is a single-centre, open-labelled RCT conducted at the inborn NICU of a tertiary care hospital in Telangana, India, for a period of 6 months. Approval of the institutional ethical committee was obtained prior to the commencement of the study. Written and informed consent was obtained from parents during enrolment. Preterm infants with birth weight between 1,000 and 1,500 grams (g) and gestational age < 34 weeks were eligible for participation. Neonates who expired or were discharged against medical advice prior to randomization were excluded. Other criteria for exclusion were neonates with congenital anomalies, neonates who received formula feeds at any point of time during the study, very sick neonates with failure of initiation of enteral feeds within 5 days of birth, and neonates whose parents did not consent for their participation in the study. Assessment of growth velocity was the primary outcome. Secondary outcomes taken into consideration were feed intolerance, NEC, sepsis and duration of hospital stay.

During the study period, all the neonates were given homogenous care with respect to clinical practice, feeding protocol, management of respiratory or other morbidity and treating faculty. All the infants were initiated on trophic enteral feeds once they were hemodynamically stable. Only MOM or PDHM was used for feeding these infants. Feeds were advanced at 10-20 ml/kg/day. When the neonates reached 100 ml/kg/day of enteral feeds, intravenous fluids were discontinued, and they were randomized to receive either HMBF or BMBF for fortification. Randomization was done using a computer-generated random sequence. "Lactodex HMF" (Raptakos, Brett & Co, Ltd, India) was the BMBF used, and it provided 0.27 g of protein and 3.37 kcal per 1 g of the powder, in contrast to the HMBF "Neolact MMF" (Neolacta, India) which provided 0.12 g of protein and 3.5 kcal per 1 g of liquid. As the BMBF used was in powdered form and HMBF used was in liquid form, blinding could not be done. One gram of fortifier was mixed with 25 ml of human milk. Enteral feeding and proportionate fortification were gradually increased until the infant reached a volume of 180 ml/kg/day. Fortification was continued until discharge. All the infants also received probiotics (a combination of *Lactobacillus spp.* and *Bifidobacterium spp.*) for NEC prophylaxis, along with calcium, phosphorus and iron supplementation as per unit protocol. Neonates in both groups were observed for weight gain and growth velocity until discharge.

Data was collected from the individual case records. Nutritional assessment of preterm infants was done by serial measurements of weight daily, head circumference and length weekly. Data was recorded by third persons who were not involved in the study. Other variables noted were the number of feeding interruptions, feed intolerance, NEC and sepsis.

In our unit protocol, the abdominal girth at the level of the umbilicus is used to objectively measure the abdominal distension. Any increase > 2 cm is taken as a significant value, and such babies were closely monitored for pre-feed aspirates and other signs of NEC.

For the purpose of the study, the following definitions were considered:

1. feed interruption: withholding enteral feeds for more than 6 hours;
2. feed intolerance: abdominal distension with increased abdominal girth > 2 cm, or significant pre-feed gastric residual > 50%, or pre-feed gastric residual with altered colour amounting to 25% of the feed.

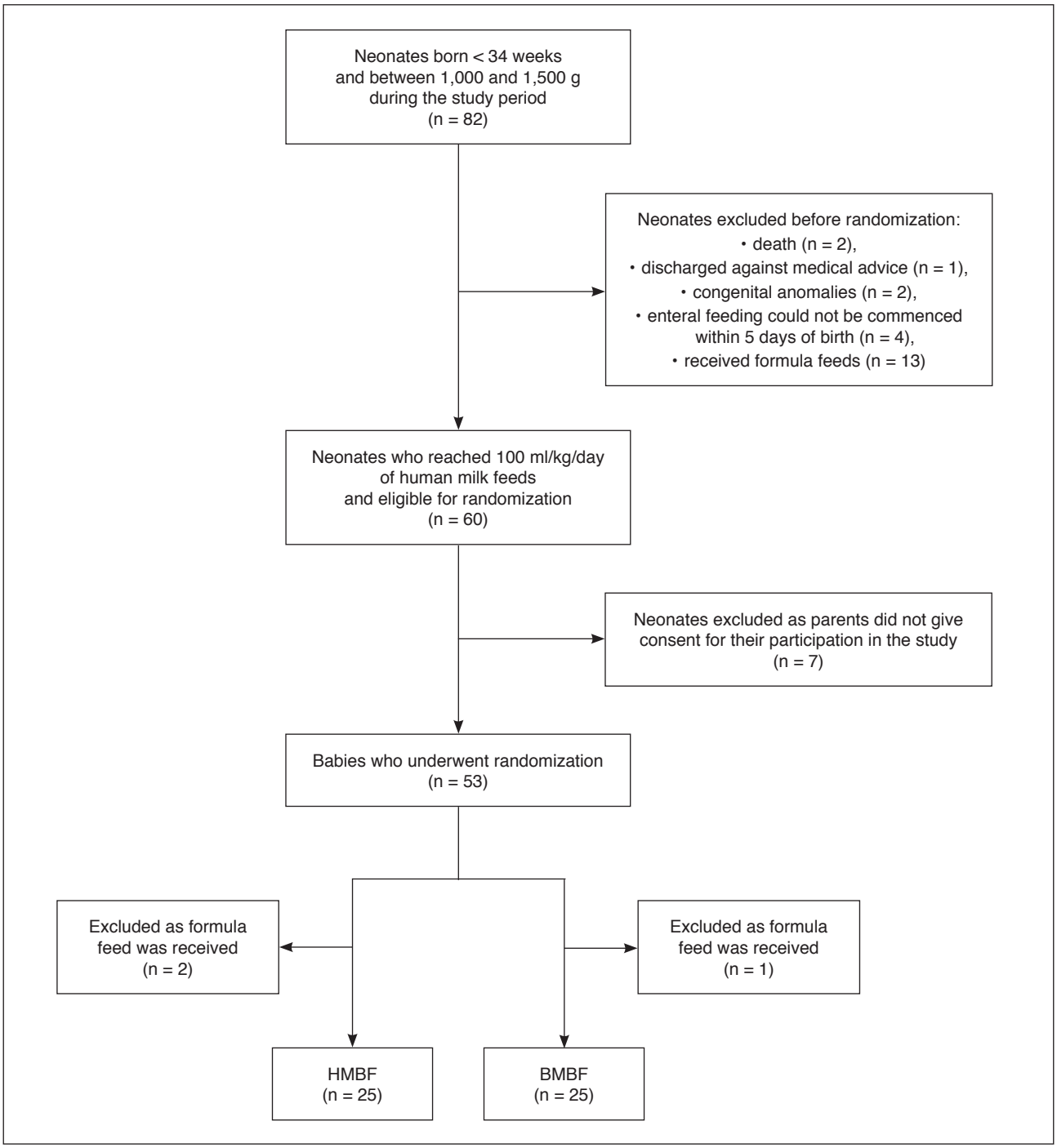
NEC was defined as stage ≥ 2 of modified Bell's classification, and sepsis was considered when any body fluid showed growth of an organism (culture-positive sepsis). Fenton's preterm growth chart was used for plotting anthropometry. SGA (small for gestational age), AGA (appropriate for gestational age) and LGA (large for gestational age) were considered when the birth weight fell < 10^{th} centile, between 10^{th}-90^{th} centile and > 90^{th} centile on the growth charts, respectively.

Statistical analysis was done using IBM® SPSS® statistics software, v. 21. Categorical variables were analysed by Chi-square test, and quantitative variables were analysed by unpaired t-test. P-values less than 0.05 were considered statistically significant. All statistical analyses were carried out at a 5% level of significance, and p-value < 0.05 was considered significant. The sample size in each group was calculated by Epi Info™ as 25 with alpha error 0.05 and power 80.

Results

The flow diagram of the study population is depicted in **Fig. 1**. Each intervention arm received

Figure 1. CONSORT diagram of the study population.



HMBF: human milk-based fortifier; BMBF: bovine milk-based fortifiers.

25 neonates. Baseline characteristics of the study population are compiled in **Tab. 1**. Neonates in both arms were comparable with respect to their baseline characteristics. Primary outcome (growth) and secondary outcomes (feed intolerance and morbidity) are depicted in **Tab. 2** and **Tab. 3**, respectively. Weight gain (21.42 vs. 20.84 g/day, p = 0.77) and growth velocity (16.45 vs. 15.85 g/kg/day, p = 0.57) were similar in both groups with no statistical difference. Sepsis (relative risk [RR] = 0.6), feed intolerance (RR = 0.57), NEC (RR = 0.33) and median duration of hospital stay (33 vs. 36 days) were better in the HMBF group than in the BMBF group.

Discussion

It is known that proper weight gain is essential for better neurocognitive development [21]. In this study, growth was observed to be similar in both groups. In fact, numerically, weight gain was

Table 1. Baseline characteristics of the study population.

Parameter		HMBF (n = 25)	BMBF (n = 25)
Birth weight (g), mean ± SD		1,294.64 ± 102.1	1,304.21 ± 101.5
Gestational age (weeks), mean ± SD		30.34 ± 2.1	30.84 ± 2.4
Growth status at birth, n (%)	SGA	8 (32%)	10 (40%)
	AGA	17 (68%)	15 (60%)
Male, n (%)		11 (44%)	9 (36%)
Maternal age (years), mean ± SD		26.4 ± 3.6	28.2 ± 4.2
Primipara mother, n (%)		17 (68%)	13 (52%)
Antenatal corticosteroids received, n (%)		16 (64%)	19 (76%)
Associated morbidity	RDS, n (%)	15 (60%)	17 (68%)
	Surfactant requirement, n	2	2
	HS-PDA [a], n	2	1
	Hyperbilirubinemia, n (%)	23 (92%)	22 (88%)
Age at initiation of fortification (days), mean ± SD		8.23 ± 2.2	9.42 ± 2.8
Weight at the initiation of fortification (g), mean ± SD		1,121.71 ± 95.6	1,264.82 ± 98.7

[a] Hemodynamically significant patent ductus arteriosus requiring medical closure.
HMBF: human milk-based fortifier; BMBF: bovine milk-based fortifier; SD: standard deviation; SGA: small for gestational age; AGA: appropriate for gestational age; RDS: respiratory distress syndrome; HS-PDA: hemodynamically significant patent ductus arteriosus.

Table 2. Growth parameters in preterm neonates receiving human milk-based fortifier (HMBF) compared to those receiving bovine milk-based fortifier (BMBF).

Parameter [a]	HMBF (n = 25)	BMBF (n = 25)	p-value [b]
Weight gain (g/day), mean ± SD	21.42 ± 6.7	20.84 ± 7.8	0.77
Head circumference gain (cm/week), mean ± SD	0.37 ± 0.21	0.37 ± 0.14	1.00
Length gain (cm/week), mean ± SD	0.32 ± 0.23	0.31 ± 0.21	0.87
Growth velocity (g/kg/day), mean ± SD	16.45 ± 3.6	15.85 ± 3.9	0.57

[a] For definitions of the outcomes – refer to methodology; [b] p-value was calculated by unpaired t-test.
HMBF: human milk-based fortifier; BMBF: bovine milk-based fortifier; SD: standard deviation.

Table 3. Feed intolerance and morbidity in preterm neonates receiving human milk-based fortifier (HMBF) compared to those receiving bovine milk-based fortifier (BMBF).

Parameter [a]	HMBF (n = 25)	BMBF (n = 25)	RR	OR
Sepsis, n (%)	3 (12%)	5 (20%)	0.60	0.55
Feed intolerance, n (%)	4 (16%)	7 (28%)	0.57	0.49
NEC, n (%)	1 (4%)	3 (12%)	0.33	0.31
Length of hospital stay (days), median (IQR 25-75)	33 (22-42)	36 (26-43)	0.0001 [b]	

[a] For definitions of the outcomes – refer to methodology; [b] p-value is significant, by unpaired t-test.
HMBF: human milk-based fortifier; BMBF: bovine milk-based fortifier; RR: relative risk; OR: odds ratio; NEC: necrotizing enterocolitis; IQR: interquartile range.

better documented in the HMBF arm (though very little) in spite of lesser protein content in HMBF, but the p-value was insignificant. A similar result was observed in the various other studies with no significant difference in growth between the two groups [22-24]. However, a recent meta-analysis published in 2020 observed lower weight gain in the HMBF group (p = 0.02), with no differences in length and head circumference [25]. In a study conducted in Italy, along with weight gain, nitrogen absorption and assimilation, and fat absorption rate were similar in both groups [23]. In another study by Polberger et al., along with growth, preprandial concentration of urea, transthyretin, transferrin and albumin were similar in both groups. In the same study, serum amino acid profile was similar in both groups except for increased threonine in neonates who received BMBF, compared to elevated ornithine and proline levels in neonates supplemented with HMBF [24].

Feed intolerance and NEC were lower in the HMBF arm with an RR of 0.57 and 0.33, respectively. Similarly, in a study by Assad et al., it was observed a significant decrease in feed intolerance and NEC with a lesser number of days to reach full enteral feeds in the HMBF group [22].

In the meta-analysis by Ananthan et al., also there was a significant reduction in the incidence of NEC in neonates who received HBMF [25].

In our study, we noticed a lower incidence of sepsis in the HMBF arm compared to the BMBF group, with an RR of 0.6. In the meta-analysis of 6 RCTs, Ananthan et al. observed no significant difference in the incidence of late-onset sepsis (LOS) in the two groups with RR of 0.96 [25]. The same study also showed a reduction in mortality in the HMBF group (RR = 0.4) without a statistically significant p-value (p = 0.09). There was no mortality in our study. In another study, a composite index of mortality and morbidity was analyzed, and they found no statistical difference between both groups [26].

The mean duration of hospital stay was less by 3 days in the HMBF group compared to the BMBF group in the present study. The major concern with the use of HMBF is its high cost. However, lesser hospitalization days and a significant reduction in the cost of hospitalization were highlighted in a study conducted by Assad et al. [22].

Strengths of the study

Being a single-center study, the feeding protocol was standardized and homogenous. Throughout the study, only human milk was used, to minimize the effect of confounding variables (formula feeds) on the results.

Limitations of the study

Limitations of the study are its small sample size, inability to perform blinding, use of only standard fortification and restriction of the study population to VLBW neonates without enrolment of extremely low birth weight (ELBW) infants due to their increased vulnerability.

Conclusion

Growth velocity was similar in neonates supplemented with either HMBF or BMBF. However, HMBF was well tolerated by neonates with lesser incidence of feed intolerance, NEC, sepsis and lesser duration of hospital stay, compared to neonates supplemented with BMBF. Given the fewer number of studies, there is a need for well-powered RCTs with a good sample size to fill the knowledge gap and draw concrete conclusions and guidelines on this topic.

Declaration of interest

The Authors declare that there is no conflict of interest.

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