

Growth and Developmental Outcomes in Infants Receiving Exclusive Breastmilk, Donor Human Milk or Mixed Feeding During the First Six Months of Life: A Prospective Cohort Study

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Abstract

Background: The first six months of life are a critical period for infant feeding and healthy growth, immune maturation and early neurodevelopment. Comparative data on infants who are fed exclusively with breastmilk, donor human milk or mixed feeding in routine postnatal care, however, are still limited.

Methods: A prospective cohort study of 170 clinically stable term and late-preterm infants followed from birth to six months in a tertiary-care maternal-child health unit. Infants were categorized into three groups: exclusive breastmilk (EBM; n=62), pasteurized donor human milk when maternal milk was not enough (DHM; n=48), and mixed feeding (MF; n=60) with breastmilk and formula. Anthropometry was measured monthly and analysed using WHO growth standards. Structured age-appropriate developmental screening was used to measure development at 6 months of age.

Results: There were no differences in baseline gestational age and anthropometry of the children. At six months, mean weight was 7.42±0.74 kg in the EBM group, 7.18±0.68 kg in the DHM group and 7.55±0.79 kg in the MF group (p=0.041). There was no significant difference between length and head circumference. Mean cognitive scores were highest in EBM infants (101.6±7.4), followed by DHM (100.4±7.2) and MF (98.2±8.1; p=0.032). The rate of any developmental concern was 3.2%, 4.2% and 11.7% of infants, respectively (p=0.046). Diarrhoeal episodes were more frequent in the MF group (23.3%) than in the EBM (8.1%) and DHM (10.4%) groups (p=0.018).

Conclusion: Exclusive breastmilk and donor human milk-supported feeding was correlated with satisfactory growth and marginally better early developmental and morbidity profile than mixed feeding. Enhanced lactation support and controlled access to donor milk could have a positive impact on early life outcomes.

Keywords: Exclusive Breastfeeding; Donor Human Milk; Mixed Feeding; Infant Growth; Neurodevelopment; Six Months; Prospective Cohort.

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Introduction

Human milk is recognized as the biological reference standard for infant nutrition due to its energy, macronutrient, micronutrient and bioactive content tailored to the infant's developmental stage. Breastfeeding has been shown to reduce infectious morbidity and mortality in the world and could provide longer-term cognitive and metabolic benefits [1]. The American Academy of Pediatrics suggests exclusive breastfeeding for about the first 6 months of life, and then breastfeeding with appropriate complementary feeding for 2 years or longer, as long as both parties desire to continue

[2]. The nutritional superiority of breastmilk is not only due to its digestible protein, lipid and carbohydrate composition, but also to its changing composition of immunoglobulins, lactoferrin, lysozyme, oligosaccharides, hormones, growth factors, enzymes and living cells. These constituents contribute to the development of mucosal immunity, maturation of the gut, development of the microbiome, and appetite regulation [3,4]. Therefore, growth assessment of infants should be compared to standards from healthy breastfed infants, not formula-fed infants

[5]. The first six months are also a critical time for brain development, sensory-motor integration and caregiver-infant interaction. Longer and exclusive breastfeeding has been linked to small improvements in cognitive, language and intelligence development, after controlling for socioeconomic and maternal factors, in both randomized and longitudinal studies [6,7]. Breastfeeding has been shown to be associated with better performance in intelligence tests by a meta-analysis, but there are residual confounding and variation in the definitions of exposure [8]. A large prospective birth cohort from Brazil also found that breastfeeding duration was correlated with intelligence, schooling and income at 30 years, suggesting that early nutrition could have a life-long impact on human capital [9].

In the absence of mother's milk or when it is not available, pasteurized donor human milk is increasingly being utilized, especially in neonatal units and human milk-bank networks. Donor human milk has many human milk-specific bioactive properties, with some immunological and enzymatic activity possibly lost due to pasteurization and donor-pool variability. In preterm or low birth weight infants, donor human milk has been shown to reduce the risk of NEC when compared to formula, but may be correlated with slower early weight gain if not fortified appropriately [10-12].

Although milk-bank services have increased, there is little prospective evidence that can be used to compare early growth and developmental outcomes for stable infants who are exclusively breastfed, fed donor human milk, and fed mixed feeds during the first 6 months of life. Previous studies in very preterm infants have shown that exposure to human milk is important for later neurodevelopment, but these studies cannot be directly applied to term and late-preterm infants in the community setting of routine follow-up [13].

The purpose of the present study was thus to compare anthropometric growth, early developmental performance and selected morbidity outcomes between infants who were exclusively breastfed, exclusively fed donor human milk and infants who were fed mixed feeding in the first 6 months of life.

Materials and Methods

Study design and setting: This was a prospective observational cohort study carried out in the postnatal ward, the newborn follow-up clinic and the lactation support unit of a tertiary care hospital for 18 months. Infants were recruited within 72 hours after birth and followed monthly until 6 months chronological age.

Study population: Singleton infants born at 34-42 completed weeks of gestation and with birth weights of 1800 g or more were eligible. Infants were excluded if they had major congenital anomalies, chromosomal disorders, congenital infections, inborn errors of metabolism, severe birth asphyxia, gastrointestinal malformations, or failed to have complete follow-up data, or if they required more than 7 days of neonatal intensive care. Mothers who had contraindications to breastfeeding were also excluded.

Grouping of participants: Feeding exposure was categorized based on the main pattern in the first six months. The exclusive breastmilk group was fed exclusively mother's milk (excluding prescribed medicines or vitamin drops). Breastfeeding support was provided and pasteurized donor human milk was provided as the main supplement when mothers own milk was not adequate. Mixed-feeding group were fed breastmilk and commercial infant formula for at least four weeks or more consecutive feeds. Maternal diaries were used to obtain feeding histories, and confirmed at monthly visits.

Sample size: With a clinically relevant difference of 0.35 in weight-for-age z-score between any two groups, a standard deviation of 0.60, 80% power and alpha error of 0.05, a minimum of 45 infants per group was needed. 190 infants were enrolled, with 180 completing the follow-up. Of the 200 infants, 170 infants were included in the analysis (62 infants in the EBM group, 48 infants in the DHM group, and 60 infants in the MF group).

Data collection: Maternal age, parity, education, mode of delivery, gestational age, birth weight, length, head circumference and early neonatal morbidity were included as baseline data. Anthropometry was taken by trained nurses using calibrated infant weighing scales, infantometers and non-stretchable measuring tapes. Weight, length and head circumference z-scores were calculated using WHO standards. Structured screening was used to evaluate development at 6 months, including cognitive, language and motor domains. Any score below 85 or failure on two age-appropriate milestones were considered developmental concern. Data on morbidity comprised diarrhoeal episodes, respiratory infections, hospitalizations and antibiotic exposure.

Data analysis: SPSS 28.0 software was used for statistical analysis. All continuous variables were presented as mean \pm SD and all categorical variables were presented as frequency and percentage. One-way ANOVA with Tukey post-hoc testing was used for normally distributed continuous variables, while the Kruskal-Wallis test was used for skewed variables. Chi-square or Fisher's exact test was applied for categorical

variables. A multivariable linear regression model adjusted for gestational age, birth weight, maternal education, parity and mode of delivery was used to examine the association between feeding group and six-month developmental scores. The p value < 0.05 was regarded as statistically significant.

Results

A total of 170 infants were followed up for 6 months. The cohort included 88 male infants (51.8%) and 82 female infants (48.2%). The baseline maternal and neonatal characteristics were generally similar between the feeding groups, except that the mean gestational age and birth weight were slightly lower in the DHM group, but not significantly (Table 1). The rate of cesarean delivery was 43.5% of EBM infants, 47.9% of DHM infants and 45.0% of MF infants (p=0.891).

All groups had satisfactory mean anthropometric progression at six months. The mean weight was significantly different between groups, with the highest mean weight in the MF group and the lowest in the DHM group (p=0.041). After post-hoc comparison, however, differences in length, head circumference and weight-for-age z-scores

were not statistically significant (Table 2). The percentage of infants whose weight-for-age was below -2 SD was 3.2% in EBM group, 6.3% in DHM group and 5.0% in MF group (p=0.728).

There were slight but statistically significant differences in cognitive and motor scores at developmental screening at 6 months. The infants fed with EBM had the highest mean cognitive and motor scores, and the infants fed with mixed feeding had the lowest scores. Any developmental concern was identified in 2 infants (3.2%) in the EBM group, 2 infants (4.2%) in the DHM group and 7 infants (11.7%) in the MF group (p=0.046). The rate of diarrhoeal morbidity was significantly higher in the MF group compared to the two human milk-dominant groups (p=0.018) and there were non-significant trends for respiratory infections and hospitalization rates (Table 3). In adjusted regression analysis, EBM exposure was associated with a 3.1-point higher cognitive score than MF exposure (95% CI: 0.6 to 5.6; p=0.016), while DHM exposure was not significantly associated with a higher cognitive score (95% CI: -0.5 to 4.6; p=0.112).

Table 1: Baseline maternal and neonatal characteristics of the study cohort

Variable	EBM (n=62)	DHM (n=48)	MF (n=60)	p-value
Maternal age (years)	27.9 ± 4.1	28.4 ± 4.6	27.6 ± 4.2	0.614
Primiparous mothers, n (%)	34 (54.8)	28 (58.3)	32 (53.3)	0.866
Maternal education ≥ secondary, n (%)	47 (75.8)	34 (70.8)	41 (68.3)	0.642
Cesarean delivery, n (%)	27 (43.5)	23 (47.9)	27 (45.0)	0.891
Gestational age (weeks)	38.1 ± 1.2	37.5 ± 1.5	37.8 ± 1.3	0.067
Male sex, n (%)	32 (51.6)	24 (50.0)	32 (53.3)	0.940
Birth weight (kg)	2.93 ± 0.37	2.78 ± 0.42	2.86 ± 0.39	0.082
Birth length (cm)	49.1 ± 1.8	48.6 ± 2.0	48.8 ± 1.9	0.346
Birth head circumference (cm)	33.8 ± 1.1	33.5 ± 1.2	33.7 ± 1.1	0.421

EBM: exclusive breastmilk; DHM: donor human milk; MF: mixed feeding. Values are mean ± SD unless otherwise stated.

Table 2: Anthropometric outcomes at six months according to feeding pattern

Outcome at 6 months	EBM (n=62)	DHM (n=48)	MF (n=60)	p-value
Weight (kg)	7.42 ± 0.74	7.18 ± 0.68	7.55 ± 0.79	0.041
Mean weight gain (g/day)	25.0 ± 4.1	24.3 ± 3.9	25.6 ± 4.3	0.048
Length (cm)	65.8 ± 2.3	65.2 ± 2.1	65.6 ± 2.4	0.210
Head circumference (cm)	42.7 ± 1.3	42.4 ± 1.2	42.5 ± 1.4	0.370
Weight-for-age z-score	-0.04 ± 0.71	-0.18 ± 0.66	0.09 ± 0.75	0.058
Length-for-age z-score	-0.02 ± 0.63	-0.13 ± 0.59	-0.06 ± 0.65	0.652
Weight-for-length z-score	0.06 ± 0.69	-0.05 ± 0.64	0.18 ± 0.72	0.143
Weight-for-age < -2 SD, n (%)	2 (3.2)	3 (6.3)	3 (5.0)	0.728

Z-scores were calculated using WHO child growth standards. Values are mean ± SD unless otherwise stated.

Table 3: Developmental and morbidity outcomes at six months

Outcome	EBM (n=62)	DHM (n=48)	MF (n=60)	p-value
Cognitive score	101.6 ± 7.4	100.4 ± 7.2	98.2 ± 8.1	0.032
Language score	100.8 ± 7.1	99.6 ± 7.5	97.9 ± 8.0	0.074
Motor score	101.9 ± 6.8	100.2 ± 7.0	98.6 ± 7.4	0.028
Any developmental concern, n (%)	2 (3.2)	2 (4.2)	7 (11.7)	0.046
At least one diarrhoeal episode, n (%)	5 (8.1)	5 (10.4)	14 (23.3)	0.018
Respiratory infection, n (%)	9 (14.5)	8 (16.7)	15 (25.0)	0.142
Hospitalization, n (%)	2 (3.2)	2 (4.2)	6 (10.0)	0.181
Antibiotic exposure, n (%)	6 (9.7)	6 (12.5)	13 (21.7)	0.167

Developmental scores are standardized domain scores with mean 100 and SD 15. Values are mean ± SD unless otherwise stated.

Discussion

In the present prospective cohort study, infants who were exclusively breastfed, those who were fed donor human milk-supported feeding and those who were fed mixed feeding had acceptable mean growth at 6 months. The mean weight and daily weight gain were slightly higher in the mixed-fed infants, while cognitive and motor scores were marginally better in exclusive breastmilk infants and diarrhoeal illness rates were lower. Infants fed donor human milk had growth and developmental outcomes similar to infants fed exclusively with breastmilk but better than infants fed mixed feeds, indicating that human milk exposure may continue to be beneficial in infants fed partially with pasteurized donor milk.

The increase in mean weight in the mixed-feeding group should be interpreted with caution. Formula supplementation may increase energy and protein intake, leading to greater early weight gain. But fast infant weight gain does not necessarily equal good growth, especially if linear growth and head circumference are comparable for all feeding groups. The WHO standards were specifically designed from healthy breastfed populations and focus on physiological growth, not maximum weight gain [5]. Our findings thus suggest that ongoing monitoring should be encouraged instead of routine formula feeding for weight gain.

The lower diarrhoeal morbidity rates in the EBM and DHM groups are biologically plausible. Pathogen exclusion and immune maturation is provided by human milk oligosaccharides, secretory IgA, lactoferrin and other antimicrobial factors [3,4]. The pattern of NEC and morbidity has been consistently lower in infants fed human milk than in infants fed formula in systematic reviews in the preterm and low-birth-weight populations [10-12]. Our cohort was not made up of very-low-birth-weight infants, but the direction of association is consistent with the overall protective effect of human milk on early gastrointestinal health.

There were small but clinically significant developmental differences at six months. After adjustment, exclusive breastmilk exposure was correlated with about three more score points on the cognitive scale. This size is comparable to the cognitive benefits found in earlier studies for breastfeeding length and exclusivity [6-9] that were small but significant. These may involve long-chain polyunsaturated fatty acids, choline, sphingomyelin, hormones, microbiome-mediated immune signaling and increased mother-infant interaction. Human milk exposure has been linked to better brain development and neurocognitive outcomes in preterm cohorts, further highlighting the significance of human milk during critical windows of neural maturation [14].

The DHM group was not identical to the EBM group, which may be due to differences in maternal-infant dyadic interaction, donor milk processing, nutrient variability and baseline neonatal vulnerability. However, recent studies in low-risk or moderate to late preterm infants have shown that donor milk supplementation does not necessarily demonstrate superiority over formula for all short-term outcomes, especially when given for a short duration [15,16]. However, donor milk is still a valuable option when maternal milk is not available, particularly when paired with intensive lactation support and personalized nutrition monitoring.

Our results are consistent with previous randomized studies that found that mother's milk was linked to fewer infection-related outcomes than donor milk or preterm formula in extremely premature infants [17]. They also corroborate the overall finding of the Cochrane review on exclusive breastfeeding duration, which did not provide consistent evidence of growth deficit with exclusive breastfeeding up to 6 months, but did provide evidence of protection against gastrointestinal infection [18]. The clinical teams should not consider donor human milk as a substitute for mother's milk, but rather as a complementary milk in the context of a hierarchy that prioritizes mother's milk, followed by donor milk when clinically indicated, and finally by formula when human milk is not available, not enough or not suitable.

The strengths of this study are that it is prospective, is followed up monthly, and uses a standard anthropometric measurement and simultaneous evaluation of growth, development and morbidity. Some restrictions should be noted, however. Exposure to feeding was not randomized and residual confounding due to maternal motivation, socioeconomic factors and health-seeking behavior is possible. Assessment of development was done at six months using a screening instrument instead of a full diagnostic battery of neurodevelopmental tests. No biochemically analysis of donor milk composition or exact volumes were performed. Lastly, the follow-up period was six months only, and a longer period of follow-up is required to see if early developmental differences continue through later infancy and childhood.

Conclusion

Infants fed exclusively with breastmilk and donor human milk had satisfactory growth in the first 6 months, and had lower diarrhoeal morbidity and modestly higher early developmental scores than those fed with mixed feeding. There were no benefits of mixed feeding for length, head circumference or developmental screening results, but slightly higher weight gain was seen. In environments where mothers' milk supply is delayed or inadequate, strengthening breastfeeding counselling, early lactation support and safe access to donor human milk may help optimize infant growth and developmental health.

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