Original Article

Effect of fortification of breast milk on the growth of preterm neonates

Geeta Gathwala[a], Chandan Kumar Shaw[b,*], Prachi Shaw[c], Rajesh Batra[a]

[a]*Department of Pediatrics, Pt. B.D. Sharma Postgraduate Institute of Medical Sciences (PGIMS), Rohtak, India.*
[b]*Department of Pediatrics, SMVMCH, Pondicherry, India.*
[c]*Department of Microbiology, SMVMCH, Pondicherry, India.*

Abstract. Human milk fortifiers (HMF) make up for the nutrient deficit required by preterm, low birth weight neonates. With the availability of a new HMF in India, it was pertinent to test this nutritional advantage in the Indian preterms fed with fortified human milk in terms the bioavailability of the important nutrients. METHODS: Sixty preterm neonates were randomly assigned in two groups of comparable gestational age and weight, the first fed with breast milk fortified with Lactodex fortifier and the second fed with EBM only. The changes in the mean levels of serum total protein and electrolytes (calcium, sodium, potassium (Na^+, K^+) and phosphorus), and blood urea in the two groups after two weeks were compared using the student's t - test. RESULTS: After two weeks of fortification the mean total serum protein (study group 5.65±0.27 gm/L compared to 5.39±0.25 gm/L in the control group, p<0.001), serum calcium (study group at 9.24±0.32 mg/dl compared to 8.87±0.25 mg/dl in the control group p<0.001), phosphate (5.54±0.18 mg/dl in the study group versus 5.31±0.24 mg/dl in the control group, p<0.001) and Na^+, K^+ levels were significantly higher than the control group. The fortification was well tolerated. We concluded that using the new fortifier not only provides all the advantages of breast milk but also provides higher amounts of proteins and calcium necessary for sustaining growth and achieving intra-uterine accretion rates in the preterm neonates and is well tolerated.

Key words: Preterm neonates and human milk fortifiers.

1. Introduction

Human milk is the best source of nutrition for newborn babies and its advantages relating to improvement in host defenses, digestion and absorption of nutrients, gastrointestinal functions and neurodevelopment are universally accepted. The premature infant is unique, however, in that feeding intolerance and frequent need for fluid restriction must be considered so that adequate nutrition can be provided to meet the needs for sustaining intrauterine rates of growth and nutrient accretion.

*Correspondence: Dr. Chandan Shaw
Assoc. Professor, Dept. of Pediatrics,
Sri Manakula Vinayaga Medical College and Hospital. (SMVMCH), Pondicherry, India
Tel: +918870722212.
E-mail: chandan.1974@gmail.com
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The exclusive feeding of unfortified human milk in premature infants has been associated with poorer rates of growth and nutritional deficits during and beyond hospitalization. Further more, the premature infant usually is tube fed making ad-libitum feeding unlikely and more often than not, fluid restriction often is imposed on their clinical management. Inadequacies of both macro- and micronutrients in preterms have adverse outcome in their overall prognosis. These inadequacies can be improved by fortification of human milk as it provides the benefits of human milk and improved nutrient intake to meet the increased nutritional needs of a rapidly growing preterm. With a Human Milk Fortifier (Lactodex HMF, Raptakoss Brett) being made available in India, the nutritional advantage of fortified breast milk is now available to the Indian neonate. The present study was planned to assess this nutritional advantage conferred to the Indian preterms fed with fortified human milk.

2. Materials and methods

The study was conducted in the Department of Paediatrics, Pt. B.D. Sharma PGIMS, Rohtak. Fifty consecutive preterm infants delivered between 1st May, 2002 and 31st December, 2002 and admitted to the Neonatology Unit, Department of Paediatrics, Pt. B.D. Sharma PGIMS, Rohtak and fulfilled the selection criteria formed the subjects for study.

Healthy preterm appropriate for gestation age neonates, without any birth asphyxia, on breast feeds / expressed breast milk having birth weight less than 1800 grams were included in the study. Small for gestation age neonates, those with congenital malformations including congenital heart disease, those not on enteral feeds by 14 days of life or preterms in whom more than 25% of daily requirement of milk needed to be supplemented with formula/another milk were excluded from the study. Neonates needing oxygen therapy for more than 10 days, ventilatory support for more than 7 days of life or diuretic/steroid therapy were also excluded as were babies with chronic lung disease.

A total of 50 preterm infants were studied. They were randomly assigned either to the control group (fed unfortified breast milk) or the study group (fed fortified breast milk) using the random number table. The study group received breast milk fortified with Human Milk Fortifier (HMF), Lactodex HMF. Human Milk Fortifier was added to the expressed breast milk, which was collected from mother each time before feed. Fortification of breast milk was done when baby started accepting 100 ml/kg/day of enteral feeds (one sachet Lactodex HMF/50 ml EBM). The fortified milk intake was maintained at the same volume for a period of 2 days after fortification was started and then gradually hiked as tolerated till a weight gain of 15 grams/kg/day was achieved. Special preterm formula (Lactodex LBW) was given to fulfill the requirement of the baby in case where the mother's milk was unable to meet the daily requirement. In case the patient could not afford the formula fed, another milk was used for the same. The cases were dropped from the study if requirement of formula feed was more than 25% of total daily requirement. Babies in the control group received EBM with no fortification. Babies in both the groups were managed according to the unit protocol other than the fortification of milk. Details regarding antenatal history and socio-economic status of the family were recorded along with weight, length, occipitofrontal circumference (OFC), gestational age, apgar score and sex of the baby. Abdominal distension, vomiting/possetting, stool frequency, volumes of gastric aspirates, if any, was also recorded. The duration of hospital stay, the time taken to regain birth weight and the time taken to achieve a weight of 2200 grams were also recorded. Biochemical markers of nutritional status - serum proteins, blood urea, serum electrolytes (Na^{+}, K^{+}), serum calcium and phosphate and serum alkaline phosphatase were estimated at the time of entry into the study and then every two weekly till the time that a weight of 2200 grams was achieved.

The unpaired student's t-test was used for statistical data analysis.

3. Results

All preterm babies were less than 36 completed weeks of gestation and were appropriate for gestation age and were apparently well, none had perinatal asphyxia and had minimum 1 minute and 5 minute Apgar score 7. Sixty seven babies met the selection criteria and were randomly assigned to the study (n=34) and control (n=33) group using the random number table. Seven babies (4 in the study group and 3 in the control group) needed supplementation with another milk, which was more than 25% of total daily requirement in the study and control groups, and were therefore excluded. In the final analysis there were 30 babies in the study group and 30 babies in the control group. In study group, 17 were males (56.7%), while in control group 14 were males (46.7%). The mean age at the start of study was comparable at 4.6± 1.2 days in the study and 4.96 ± 1.5 days in the control group. The mean gestational age and the various physical parameters in the study and control group were comparable and without significant statistical differences. (Table 1). The subjects in both the study and the control groups reached enteral feeds of volume 100 ml/kg/day and full enteral feeds were reached at comparable days of life. Even the mean supplemented volumes in both groups were comparable (Table 1). there was no statistical difference between the episodes of possetting/day as well as the percentage of gastric aspirates of the total feeds/day between the fortified and the unfortified groups, thereby, implying that fortification was well tolerated.

Table 2 summarises the comparison of the biochemical parameters of nutrition between the two groups. All the parameters were comparable at the beginning of the study, however, after two weeks of fortification the mean total serum

Table 1. Comparison of the patient parameters and the feed characteristics between the fortified and unfortified groups

Parameters	Study group (n= 30) [EBM+MHF] (mean ± S.D)	Control group (n= 30) [EBM] (mean ± S.D	P value
Sex ratio	1.31:1	0.88:1	-
Gestational age at entry	33.38 ± 1.38	32.83 ± 1.17	p>0.05
Birth Wt.	1670 ± 129	1658 ± 140	p>0.05
Length	42.26 ± 1.03	41.20 ± 1.14	p>0.05
OFC∗	29.28 ± 1.14	29.28 ± 0.88	p>0.05
$T_{f\ 100}$† (Days)	3.84 ± 0.85	4.08 ± 1.08	p>0.05
$T_{f\ full}$‡ (Days)	7.96 ± 1.34	8.32 ± 1.25	p>0.05
V_{max} (mL) ¶	171.6 ± 8.0	168.6 ± 5.3	p>0.05
% supplement of total feed/day	4.75 ± 3.33	5.41 ± 3.92	p>0.05
Possetting episodes/day	0.48 ± 0.82	0.49 ± 0.84	p>0.05
% aspirates of total feed/day	0.82 ± 1.52	0.72 ± 1.44	p>0.05

∗ OFC : Occipitofrontal circumference.
† T f 100 : Average number of days to achieve 100 ml/ Kg/ day feeds.
‡ T f full : Average number of days to achieve maximum feeds.
¶ V max : Volume of maximum feeds.

protein (study group 5.65 ± 0.27 gm/L compared to 5.39 ± 0.25 gm/L in the control group p<0.001), serum calcium (study group at 9.24 ± 0.32 mg/dl compared to 8.87 ± 0.25 mg/dl in the control group p<0.001), phosphate (5.54 ± 0.18 mg/dl in the study group versus 5.31 ± 0.24 mg/dl in the control group, p<0.001) and electrolytes (Na^+, K^+) (p<0.05) levels were significantly higher than the control group. The levels of blood urea and serum alkaline phosphatase were not significantly different between the two groups.

4. Discussion

Human milk fortifiers are of great nutritional value to preterm neonates. They supplement the micro and macronutrients lacking in the “mature” milk of mothers with preterm babies, leading to improved growth (1-3**)**, neurodevelopment (4**)** and final survival. However the there is a need to substantiate the improved growth patterns by biochemical markers of nutrition. Our study underlines the significantly higher positive nitrogen balance in fortified EBM fed preterms compared to the controls as is evident from the higher levels of serum proteins and blood urea. The presence of higher electrolyte levels in the fortified group also validates the use of HMF. Significantly higher serum total protein have been consistently reported by many authors like Mileur et al**,** (5) Hayashi et al (6), Carey et al (7) and Modanlou et al (8) using Enfamil Human Milk Fortifier (Mead Johnson Nutritionals, Evansville, Indiana, U.S.A). Some authors have studied the amino acid profile in the two groups. Kashyap et al not only demostrated a higher mean plasma albumin concentration in fortified group but also significantly higher levels of several aminoacids like threonine, valine, methionine, tryptophan, aspartate and alanine in the supplemented group (9). However Lucas et al (4) reported lower values of plasma amino acids like taurine, proline, glycine, and phenylalanine but not histidine in the fortified group (Enfamil Human Milk Fortifier, Mead Johnson Nutritionals, Evansville, Indiana, U.S.A.) compared to the unfortified group (10). Polberger et al compared fortification with a bovine whey protein and ultrafiltrated human milk protein and found similar aminoacid profiles in both the groups,

Table 2. Comparison of the biochemical parameters between the study and the fortified group

S. N.	Biochemical parameter	Mean levels at start		Mean levels after two weeks		'p' value
		Fortified group	Control group	Fortified group	Control group	
1.	Ser. Total protein (g/dL)	4.99 ± 0.28	5.07 ± 0.28	5.65 ± 0.27	5.39 ± 0.25	<0.001
2.	Blood urea (mg/dL)	23.21 ± 3.79	21.68 ± 4.07	22.80 ± 2.65	21.12 ± 2.77	>0.05
3.	Ser. Sodium (mEq/L)	135.42 ± 4.61	135.81±4.52	140.87±3.82	137.68±2.39	<0.05
4.	Ser. Potassium (mEq/L)	3.82 ± 0.55	3.73 ± 0.45	4.23 ± 0.37	4.02 ± 0.35	<0.05
5.	Ser. Calcium (mg/dL)	8.52 ± 0.22	8.58± 0.25	9.24±0.32	8.87 ± 0.25	<0.001
6.	Ser. Phosphate (mg/dL)	4.91 ± 0.21	4.93 ± 0.22	5.54 ± 0.18	5.01 ± 0.24	<0.001
7.	Ser. Alkaline phosphate (K.A.U.)	9.76 ± 1.22	9.84 ± 1.59	10.01 ± 1.66	10.12 ± 1.39	>0.05

except for threonine (significantly higher in the bovine group) and proline and ornithine (significantly higher in the human milk protein group)(11).

In the presence of normal renal functions higher levels of blood urea is indicative of a positive nitrogen balance as was observed in our study group though not statistically significant. Similar observations have been reported by others (9-12). Modanlou et al studied the effect of fortification on biochemical markers such as blood urea nitrogen and showed a highly significant rise in blood urea nitrogen, i.e. 6.7 ± 4.0 mg/dL in fortified group in comparison to 3.1 ± 0.7 mg/dL in control group after 3-4 weeks into study (8). The increased levels of urea were observed only after a period of 3 - 4 weeks by most of the others while in this study we observed the same effect in 2 weeks. However Warner et al found a significant fall in plasma urea from a median (range) of 3.0 (0.8-5.9) mmol/L at week 1 to 1.9 (0.1-7.7) mmol/L at week 3 ($p<0.05$) and 1.2 (0.9-2.8) mmol/L at week 5 ($p<0.05$) in fortified group. (13**)** The reason for the latter may the different HMF (Eoprotin) used by them.

We documented significantly higher values of electrolytes (Na and K) a finding only observed by Reis B. B. et al. (14). However most of the others did not document any significant differences in the latter (8,9,11). We observed that the serum calcium and phosphate levels were significantly higher in the fortified group, without any significant difference in the serum alkaline phosphatase (ALP) levels. Serum ALP is a marker of bone turn over or osteolysis and shows an inverse relation with the serum phosphorus levels. Thus our observation may be interpreted as an indicator of better osteogenesis and mineralization. Among other authors Modanlou et al had demonstrated identical findings (8).

Table 3. Comparison of different human milk fortifiers (prepared per 100 ml EBM)

	PrHM*	EHMF†	SNC‡	Eoprotin	SMAHMF§	FM85‖	Lactodex HMF
Energy (Cal)	71	85	76	85	86	89	80
Fat (g)	3.6	3.6¶	4	3.6¶	3.6¶	3.6	3.8
Carbohydrate(g)	7	9.7	7.8	9.8	9.4	10.6	9.4
Protein (g)	1.8	2.5	2	2.6	2.8	2.7	2.2
Calcium (mg)	22	112	97	72	112	73	122
Phosphorus(mg)	14	59	50	48	59	48	64
Magnesium(mg)	2.5	3.5	6.3	5.3	4	4.5	10.5
Sodium (mEq)	0.7	1	1.1	1.9	1.1	1.9	2.22
Zinc (μg)	320	1030	760	320¶	450	320¶	680
Copper (μg)	60	122	130	60¶	60¶	60¶	130
Vitamins	Yes¶	Multi**	Multi**	A,C,E,K	Multi**	Yes¶	Multi**

* PrHM = Premature human milk.
† EHMF = Enfamil Human Milk Fortifier (Mead Johnson Nutritionals, Evansville, IN).
‡ SNC = Similac Natural Care (Ross Labs, Columbus, OH): mixed 1:1 (vol:vol) with PrHM.
§ SMA Human Milk Fortifier (Wyeth Nutritionals International, Philadelphia, PA).
|| FM85 (Nestle, Vevey, Switzerland).
Yes = Nutrients as much as in breast milk.
¶ Indicates nutrient not contained in fortifier.
** Multivitamins:A, D, E, K, B1, B2, B6, B12, C, niacin, folate, panthothenate and biotin.

Hayashi et al observed significant increases in calcium and phosphorus levels with significantly lower levels of serum ALP probably explained by higher turnover rates of bone (12). Variable results of calcium, phosphorus and ALP have been reported by others like Carey et al (7) and Lucas et al (10) while Warner et al (13) showed no significant differences. A recent study by Zuppa et al (1) detected higher level of serum phosphorus in spite of significantly lower intakes of phosphorus occurred in fortified human milk fed neonates, as if there was a better availability of this nutrient in human milk. S.J. Gross (15) compared the effects of unfortified EBM, fortified EBM, and formula feeds and found that there was no variation in the calcium levels but the phosphorus levels were significantly higher in only the formula fed preterms after 3 weeks. However at 44 weeks postconceptional age the levels of phosphorus were uniformly high regardless of the previous diet. The ALP levels followed an inverse relation with the phosphorus levels throughout. It may be thus inferred that the effect of fortification may be over a limited time frame till the babies reach some amount of maturity by which time their nutrient intakes may compensate for the effects of fortification. Thus it may be necessary to study the effects of fortification over a longer period of time. The extremely variable results may be because of the complex interplay of several factors regulating calcium balance in the preterm neonate, not to undermine the effect of different composition of the fortifiers (Table 3), the volumes of feeds and the various time frames (maturity of the subjects and the duration) of the studies. A recent study with a iron-fortified HMF concluded that though the latter did not alter the prevalence of anemia of prematurity, it did however significantly lower the need for blood transfusions, without any effect on feed tolerance (16). The HMF we used was however not iron-fortified, nevertheless, it was well tolerated. The effect of fortification on several other biochemical markers like transthyretin, transferrin and albumin (11) and its effect on bone mineralization using photon absorptiometry (5, 15) or x-ray micro-densitometry (12) has been effectively utilized by various authors also depict the far reaching benefits. Nevertheless, the parameters used in the present study clearly depict the significant advantage of HMF Lactodex on the biochemical markers of nutrition in Indian preterms. Given the risks and cost limitations of parenteral nutrition, in a country like India (and other developing nations), we suggest that preterm neonates once tolerating a 100 ml/Kg/day feeds must be supplemented with human milk fortifiers to help them attain the intra-uterine accretion rates as well as not deny them the natural benefits of mother's milk!

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References

1. Zuppa AA, Girlando P, Scapillati ME, Maggio L, Romagnoli C, Tortorolo G. Effects on growth, tolerability and biochemical parameters of two different human milk fortifiers in very low birth-weight newborns. Pediatr Med Chir 2004; 26: 45-49.
2. Porcelli P, Schanler R, Greer F, et al. Growth in human milk-Fed very low birth weight infants receiving a new human milk fortifier. Ann Nutr Metab 2000; 44: 2-10.
3. Reis BB, Hall RT, Schanler RJ, et al. Enhanced growth of preterm infants fed a new powdered human milk fortifier: A randomized, controlled trial. Pediatrics 2000; 106: 581-588.
4. Lucas A, Fewtrell MS, Morley R, et al. Randomized outcome trial of human milk fortification and developmental outcome in preterm infants. Am J Clin Nutr 1996; 6: 142-151.
5. Mileur LM, Chan GM, Gill G. Evaluation of liquid or powdered fortification of human milk on growth and bone mineralization status of preterm infants. J Pediatr Gastroenterol Nutr 1992; 15: 370-374.
6. Hayashi T, Takeuchi T, Itabashi K, Okuyama K. Nutrient balance, metabolic response, and bone growth in VLBW infants fed fortified human milk. Early Hum Dev 1994; 39: 27-36.
7. Carey PE, Rowe JC, Goetz CA, et al. Growth and phosphorus metabolism in premature infants fed human milk, fortified human milk, or special premature formula. Am J Dis Child 1987; 14: 511-515.
8. Modanlou HD, Lim MO, Hansen JW, Sickles V. Growth, biochemical status, and mineral metabolism in very low birth weight infants receiving fortified preterm human milk. J Pediatr Gastroenterol Nutr 1986; 5: 762-767.
9. Kashyap S, Schulze KF, Forsyth M. Growth, nutrient retention, and metabolic response of low-birth-weight infants fed supplemented and unsupplemented preterm human milk. Am J Clin Nutr 1990; 52: 254-262.
10. Lucas A, Fewtrell MS, Morley R, et al. Randomized outcome trial of human milk fortification and developmental outcome in preterm infants. Am J Clin Nutr 1996; 64: 142-151.
11. Polberger S, Raiha NCR, Juvonen P, et al. Individualized protein fortification of human milk for preterm infants: Comparison of ultrafiltrated human milk protein and a bovine whey fortifier. J Pediatr Gastroenterol Nutr 1999; 29: 332-338.
12. Hayashi T, Takeuchi T, Itabashi K, Okuyama K. Nutrient balance, metabolic response, and bone growth in VLBW infants fed fortified human milk. Early Hum Dev 1994; 39: 27-36.
13. Warner JT, Linton HR, Dunstan FDJ, Cartlidge PHT. Growth and metabolic responses in preterm infants fed fortified human milk or a preterm formula. Int J Clin Prac 1998; 52: 236-240.
14. Reis BB, Hall RT, Schanler RJ, et al. Enhanced growth of preterm infants fed a new powdered human milk fortifier: A randomized, controlled trial. Pediatrics 2000; 106: 581-588.
15. Gross SJ. Bone mineralization in preterm infants fed human milk with and without mineral supplementation. J Pediatr 1987; 111: 450-458.
16. Berseth CL, Van Aerde JE, Gross S, et al. Growth, efficacy, and safety of feeding an iron-fortified human milk fortifier. Pediatrics 2004; 114:699-706.