

Efficacy of human milk fortification in low-birth-weight infants; A randomized controlled trial

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Abstract

Introduction: Low Birth Weight (LBW), less than 2500grams, has significant effect on baby survival and growth. A good supportive care in addition to breastfeeding is very important. Breast milk fortification role is under research.

Aims; To study the Efficacy of Human Milk Fortification in Low-Birth-Weight Infants

Materials and Methods: This RCT was conducted at Department of pediatrics Lady Reading Hospital, Peshawar, Pakistan, from October 2021 to March 2022. A total of 60 low birth weight neonates were randomly allocated into two arms (group A fortified) and (group B unfortified) through non probability consecutive sampling technique. One group was given fortified expressed breast milk with 1g sachet of Human Milk Fortifier (HMF). The second group was fed with unfortified breast milk only. Babies were monitored every two-weekly with Biochemical markers. Data was analyzed through descriptive and inferential means using independent T Test.

Results: The mean weight of neonates in the supplemented group was 2273.83g (Mean SD = 2273.83g 182.83g) and in the control group it was 2176.67g (Mean SD = 2176.67g 181.86g). Result shows that infants given human milk fortifier gain more weight as compared to breast milk. Statistical analysis also showed significant difference (P value 0.017)

Conclusion: It is concluded that use of human fortified milk is helpful in gaining weight in low birth weight infants. It also improves serum calcium and protein levels.

Keywords: Infants; Low birth weight; Expressed breast milk; Human milk fortification.

1. Introduction

Low Birth Weight (LBW) refers to babies who are born weighing less than 2500 grams, regardless of their gestational age.¹ This category includes two types of babies: preterm infants who are born before completing 37 weeks of gestation but are appropriately grown, and both term and preterm infants who are growth restricted and fall below the 10th percentile of weight for their gestational age and sex. The measurement of low birth weight is crucial for public health purposes, especially in settings where accurately determining gestational age can be difficult.²

This issue has far-reaching complications on human capital as it is associated with multiple short- and long-term consequences.³ LBW newborns accounts for 80% of neonatal deaths with approximately two-thirds being preterm and one-third being term small for gestational-age infants.⁴ Furthermore, LBW babies are at significant risk of morbidities and mortalities resulting in stunting and long term developmental and physical health problems including the onset of chronic diseases later on in adulthood such as cardiovascular diseases.^{5,6} Various factors influence LBW babies including

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extremes of maternal ages (especially below 16 years and above 40 years of age, multiple pregnancies, certain maternal conditions such as diabetes, hypertension, infections and nutritional status).^{7,8} Other factors that contribute to low birth weight include exposure to environmental factors such as indoor air pollution, tobacco use and substance abuse.⁹ It is worth noting that the nutritional composition of breast milk from mothers who give birth prematurely is comparable to that of mothers who deliver full-term infants, usually within about 3-4 weeks after delivery.¹⁰ Therefore, the higher nutritional requirement of a preterm newborn cannot be adequately fulfilled by unfortified breast milk.¹⁰ Breast milk naturally contains about 260mg/L calcium and 140 mg/L phosphorus.¹¹ regardless of the increased feeding rate for LBW infants at 200mL/kg, the unfortified milk only provides 50mg/kg/day calcium.¹²

Assuming a maximum absorption rate of 60% to 70%, this would still only supply about one-third of the calcium and phosphorus absorption levels experienced in the womb ¹². Adding extra nutrients to breast milk through fortification can help prevent mineral deficiencies and reduce the risk of osteopenia, while meeting the metabolic needs of LBW infants, who undergo rapid growth.¹² A study conducted by Gathwala et al. revealed that in low birth weight infants, the mean serum total protein level was 5.65 ± 0.27 g/dL in the fortified group, compared to 5.39 ± 0.25 g/dL in the unfortified group after a two-week period. Additionally, the study found that the serum calcium level was 9.24 ± 0.32 mg/dL in the fortified group, while it was 8.87 ± 0.25 mg/dL in the unfortified group ¹³. Prior to this study, no research had been conducted on the Pakistani population regarding this matter. Furthermore, there has been a lack of World Health Organization (WHO) guidelines specifically addressing the feeding of low-birth-weight infants. Unfortunately, the quality of care provided to these infants in low- and middle-income countries are often inadequate, resulting in insufficient breastfeeding or inadequate breastfeeding practices during the crucial early hours and days of life. Therefore, the objective of this study is to compare the levels of biochemical markers in low-birth-weight infants who receive fortified human milk with those who receive unfortified human milk.

2. Materials and methods

A total of 60 neonates fulfilling the inclusion criteria from Department of Pediatrics, LRH, Peshawar, was included in the study after ethical approval from Institutional Review Board of the Institution. The data was collected from 1st October 2021 to 31st March 2022, from 60 low weight birth infants, having weight less than 2500 grams at birth, admitted in pediatric department. In this study, low birth babies whose mothers were willing and able to express milk and met the inclusion criteria were included and randomly assigned to two groups. The control group, consisting of 30 children, received pure human milk. The experimental group, also comprising 30 neonates, received fortified mothers' milk.

Initially at the commencement of study, 76 children were recruited, out of which 3 were on enteral feed, 2 neonates needed oxygen therapy, 4 were started on steroid therapy and 7 lost to follow up were excluded. Babies who were on enteral feed by 14 days of life, requiring oxygen more than 10 days, ventilatory support later than 7 days of life, treated with steroids or diuretics, didn't give consent for inclusion in the study or lost follow up, were excluded. Randomization was performed by block randomization for both groups. A detailed explanation about the participation in the study was given to the parents and a verbal informed consent was obtained explaining the risks and benefits in details.

The group receiving fortified breast milk was supplemented with Human Milk Fortifier (HMF). Each time baby was breast fed, expressed breast milk was obtained and HMF was added. This supplementation of HMF to breast milk was done once baby intake was 100ml/kg/day. The volume of fortified milk intake was kept constant for two days after starting fortification and then gradually increased as tolerated until the infants reached a weight of 2200 grams. In contrast, the unfortified group was exclusively fed with unfortified breast milk until they reached a weight of 2200 grams. Both groups of these babies were managed as per our ward protocol and only modification was fortification of human milk for fortified milk fed babies.

To assess the nutritional status, the levels of biochemical markers such as serum proteins and serum calcium were measured every two-week until the infants reached a weight of 2200 grams. These measurements were recorded on a specially designed proforma.

The parameters of our study; body weight, calcium, protein level were measured both at start of the study and by the end of 1 month after human milk fortification. These parameters were compared between controlled and experimental group. Both groups control and experimental, data was described by using frequency and percentage tables along with charts, descriptive statistics, mean and standard deviation and were compared by using independent sample t-test. The analysis was carried out by using statistical package for social sciences (SPSS) version 26.

3. Results

We noted that the gender-wise distribution was 33 (55%) were male and 27(45%) were females. Their distribution in each group is shown in figure 1. The mean weight of neonates in the experimental group was 2273.83g (2.28kg) with variation from mean weight 182.23g (Mean \pm SD = 2273.83g \pm 182.83g) and in the control group it was 2176.67g (2.20kg) with variation from mean weight 181.86g (Mean \pm SD = 2176.67g \pm 181.86g). Similarly, the mean protein in the experimental group noted was 5.57g/dL with variation from mean weight 0.08g/dL (Mean \pm SD = 5.57g/dL \pm 0.08g/dL) and in the control group it was 5.34g/dL with variation from mean weight 0.08g/dL (Mean \pm SD = 5.34g/dL \pm 0.08g/dL). While average calcium measured in the experimental group was 9.07mg/dL with variation from average weight 0.15mg/dL (Mean \pm SD = 9.07mg/dL \pm 0.15mg/dL) and in the control group it was 8.65mg/dL with variation from average weight 0.24mg/dL (Mean \pm SD = 8.65mg/dL \pm 0.23mg/dL). Table1.

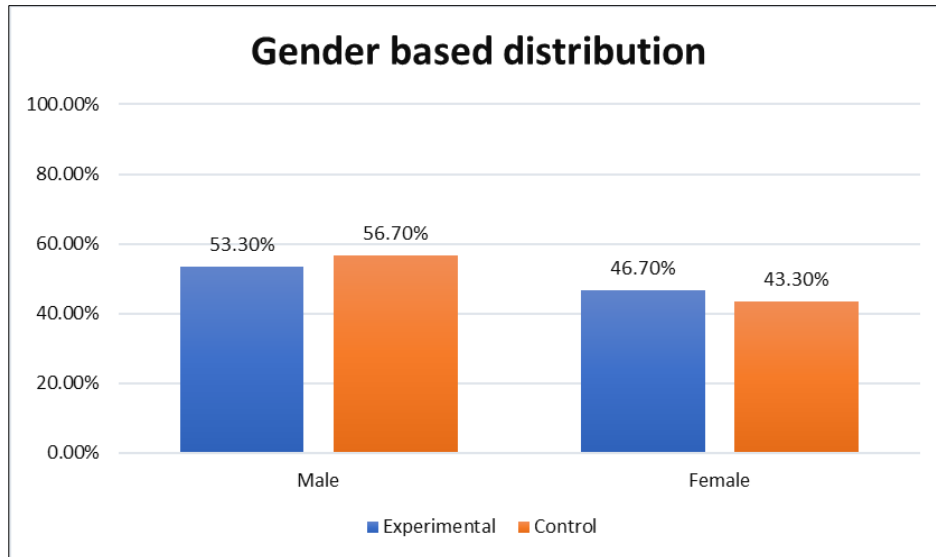


Figure 1 Gender-wise Distribution

Table 1 Descriptive statistics

Factors	Group	Number	Mean	Std. Deviation
Birth Weight (gm)	Exp.	30	2273.83	182.23
	Cont.	30	2176.67	181.86
Protein (g/dL)	Exp.	30	5.57	0.08
	Cont.	30	5.34	0.08
Calcium (mg/dL)	Exp.	30	9.07	0.15
	Cont.	30	8.65	0.24

3.1. T-test analysis

The statistical t-test was used to compare the weights, protein and calcium level in experimental and control group. It was found that, there significant difference was present in the weight of experimental and control group children, as the t-value recorded 2.445 with degree of freedom 58 and P-value of 0.017<0.05. Furthermore, statistically highly significant difference was found in the level of protein and calcium in both experimental and control groups as the t-values recorded were 11.058 and 8.037 with degree of freedom 58 and p-values 0.00 each. Table2. All the means differences were recorded positive, this shows that, in the experimental group children's weights, protein and calcium level are more compare to control group. This describes children taking mother fortified milk gain more weight, also the level of protein and calcium are high in them compared to those children taking simple mother milk.

Table 2 Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means					
	F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	95% CI of the Difference	
							Lower	Upper
Birth Weight (gm)	2.146	0.137	2.445	58	0.017	97.16	-82.421	105.754
Protein (g/dL)	0.159	0.692	11.058	58	0.000	0.233	0.19110	0.27557
Calcium (mg/dL)	2.045	0.158	8.037	58	0.000	0.423	0.31790	0.52877

4. Discussion

Human milk fortifiers (HMF) play a crucial role in providing essential micro and macronutrients to low-birth-weight neonates. This is helpful to address the nutritional deficiencies of breast milk for babies with LBW, and thus leads to improve growth of these babies, neuro-development and overall survival.¹⁴⁻¹⁷ This improvement in growth and development needs to prove with biochemical markers of nutrition, so that its significance be justified and proved. Our study highlights a significant improvement in serum protein (albumen) favoring a positive nitrogen balance in these babies who were fed fortified feeds compared to non-fortified group. Similarly presence of high normal level of serum electrolytes in fortified group further favors use of HMF in low birth weight babies. Many authors such as Reis BB et al¹⁴, Brown JV et al¹⁷, Mukhopadhyay K et al¹⁸ and Gathwala G et al¹⁹ have consistently reported significantly higher serum total protein levels with the use of HMF. Overall, these findings emphasize the beneficial effects of HMF on the nutritional status of LBW infants, as indicated by biochemical markers, and align with previous research in the field.

Several researchers have investigated the plasma amino acids profiles in these babies with fortified breast fed babies.^{20,21,22} Ahnfeldt AM et al²³ in his study found that babies with bovine colostrum fortified fed infant had improved growth parameters and had higher plasma amino acids profiles with the most pronounced for tyrosine and valine (+35%, $P < 0.001$). The measured amino acids level across all showed higher level 11% ($p < 0.001$). Combined both essential amino acids and branched-chain amino acids were increased with bovine colostrum fortified human milk fed babies. However, phenylalanine, tyrosine and tryptophan proportions were unchanged. However, Bene J et al²⁴ didn't find any difference in plasma carnitine profiles in pre-term infants fed with fortified human milk.

Polberger et al,²⁵ in his study compared bovine whey protein as milk fortifier and ultra-filtrated human milk protein and almost similar plasma amino acids profiles. However, threonine was significantly higher in bovine group and proline and ornithine was significantly higher in ultra-filtrated human milk protein group. Similar results were reported by other researchers.²⁰⁻²⁴ Gathwala G et al¹⁹ in his study also used blood urea nitrogen as biochemical markers for assessment of efficacy of fortification and found that blood urea nitrogen was significantly high in fortified group (22.80 ± 2.65 mg/dL) as compared to non-fortified/control group (21.12 ± 2.77 mg/dL) after 2 weeks.

El Sakka A et al.²⁶ conducted a study and reported no significant change in plasma urea nitrogen and albumin levels in the fortified group. His work shows that median plasma urea-nitrogen level 14.80 ± 4.76 mg/dL in fortified group and 16.36 ± 8.76 mg/dL in control group with P value of 0.44, however growth parameter shows significant improvement with weight gain of 16.8 ± 5.5 grams/kg/day in fortified group compared to 13.8 ± 4.7 0.04 grams/kg/day in control group with P value of 0.04. Similarly length gain was 0.76 ± 0.2 cm and 0.58 ± 0.19 cm with P value of 0.003 and OFC gain of 0.59 ± 0.16 cm and 0.49 ± 0.11 cm with P value of 0.02 in fortified and groups respectively. In our study, we found significantly high values of electrolytes (Na, K) which were consistent with observations of Reis BB et al¹³. It is important to mention that most of the studies didn't show any significant differences in electrolytes levels^{21, 22, 24}. We also found that the fortified group had significantly higher levels of serum calcium and protein. This observation suggests better osteogenesis and mineralization in the fortified group, supporting improved bone health. Overall, our study's findings, particularly the higher levels of electrolytes, serum calcium, and protein in the fortified group, indicate positive outcomes related to bone development and mineralization.

Enloft PR et al²⁷ demonstrated similar findings to ours. His work shows a significant improvement in Bone Mineral Content (BMC) in very low birth weight preterm infants fed with human fortified milk. Serum Alkaline Phosphatase level was significantly raised in control group. His study results shows that increase in mean BMC in fortified group was 4.90 ± 4.46 gm and in control group was 1.86 ± 3.17 gm with P value of 0.02. Similarly Serum Alkaline Phosphatase levels were 720 ± 465 IU/L in control group versus 391 ± 177 IU/L in fortified group with P value of 0.007. However, we measured only serum calcium level while he did whole-body densitometry (DEXA) and biochemical markers. His study doesn't mention plasma protein or albumin as outcome measurement used in our study. Similarly Zuppa et al¹² work shows higher serum phosphate levels for fortified breast milk fed babies. However Gulsum KS et al²⁸ didn't find satisfactory improvement in weight gain, length and head circumference in preterm infants fed with standard fortification.

Overall, these findings highlight the variability in calcium, phosphorus, and ALP levels reported in different studies, suggesting that factors such as fortification, nutrient availability, and feeding practices may contribute to these variations. Although the HMF we used in our study was not fortified with iron, it was well tolerated by the infants. Other studies have explored the effects of the human milk fortification using various biochemical markers in serum like total protein, albumin, plasma amino acids, blood urea-nitrogen and bone mineralization using different techniques^{19-24, 26, 27, 29}. These studies have demonstrated the broad-ranging benefits of fortification. Erin Grace et al³⁰ in his systematic review of the efficacy and safety of fortification of human milk for use in low birth infants and reduced incidence of necrotizing enterocolitis with human milk based fortifiers. However, the overall quality of evidence is low.

The parameters chosen in our study clearly show significant advantages of Human Milk Fortification in low birth weight babies. Looking to the risks and cost limitations associated with parenteral nutrition, specially for low income states, we suggest human milk fortification for low birth weight babies. This approach will help them to gain intrauterine growth rate and also get benefits of breastfeeding.

5. Conclusion

We found from our study that human milk fortification shows significant difference in terms of weight gain, level of protein and serum calcium compared to non-fortified breast fed low birth babies. Thus human milk fortification is a better way for low birth babies and can be safely initiated when milk intake reaches to 100ml/kg/day.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Consent was taken for all the parents of patients included in the study. Ethical and Research board of Lady Reading Hospital Peshawar gave ethical approval for this study. Permission was obtained from ethical committee of the institution for human milk fortification and measuring its effect on low birth weight babies.

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