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Effect of Protein Supplementation on the Growth of Infants Weighing Less than 1,000 Grams Hospitalized on the Neonatal Intensive Care Unit of Akbar Abadi Hospital in Tehran, Iran (2015-2016)

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ABSTRACT

Background: Breast milk provides adequate protein to facilitate growth for term infants. Appropriate nutrition is necessary for the growth of preterm infants. Extremely-low-birth-weight (ELBW) infants require higher protein intakes to achieve adequate growth.
The present study aimed to evaluate the effect of protein supplements on the physical growth of infants weighing less than 1,000 grams through the serial measurement of their anthropometric indices (weight, height, and head circumference) during admission at the neonatal intensive care unit (NICU).
Methods: This triple-blind, randomized clinical trial was conducted on 64 infants weighing less than 1,000 grams, who were admitted to the NICU of Akbar Abadi Hospital in Tehran, Iran during 2015-2016. Data on the daily nutritional intake of the subjects were recorded until discharge from the hospital. Data analysis was performed in SPSS version 24.
Results: In total, 63 infants were enrolled in the study. Mean daily weight gain of the infants was 55.92±36.90 and 30.80±13.91 grams in the case and control groups, respectively (P=0.001). Mean weekly linear growth in the case and control groups was 0.77±0.67 and 0.76±0.29 centimeter, respectively (P=0.939). Mean weekly head circumference growth in the case and control groups was 0.51±0.10 and 0.34±0.16 centimeter, respectively (P<0.001).
Conclusion: According to the results, protein therapy in the premature, extremely-low-birth-weight (ELBW) infants could improve the rate of weight gain and head circumference growth. Given the importance of weight gain in premature ELBW infants, it is recommended that protein therapy be employed in these newborns. Various studies have denoted the few side-effects of protein therapy, which indicates the safety of this method to resolve the lack of weight gain in these infants.

Keywords: ELBW infants, Growth, Protein

Introduction

Extremely-low-birth-weight (ELBW) infants are those who are delivered prior to week 37 of gestation with the birth weight of less than 1,000 grams. ELBW infants comprise 4-8% of infant population (1, 2). The survival rate of ELBW infants is significantly improved through medical interventions in neonatal intensive care units (NICUs) (2, 3). According to the American Academy of Pediatrics Committee on Nutrition,

the growth rate of ELBW infants should be similar to that of the embryos with the same gestational age by the optimum nutritional care and support (4). Despite the advancement in prenatal care and nutritional protocols (5, 6), such growth and development are not achieved in NICUs (4, 7-10). Limited postpartum growth is associated with the higher risk of neurological deficits (4, 8-11).

Concentrations of protein and other

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microelements in human breast milk are not adequate to meet the excessive requirements of ELBW infants. Growth failure is a common complication in the ELBW infants who are only breastfed (12-14). The optimal level of nutritional protein for the infants weighing less than 2,500 grams fed with formula remains controversial. The level of protein should be adequate for the optimal growth of these infants, without causing complications such as acidosis, uremia, and higher concentrations of circulating amino acids (e.g., phenylalanine) (15).

To meet the essential nutritional requirements of ELBW infants, fat-free mass weight gain should be prioritized over mere weigh gain. The ratio of fat-free body mass to the fat mass of the measured weight depends on the total protein and energy in the nutritional diet. Inadequate energy and protein intakes lead to limited weight gain and height and head circumference growth. However, if the protein intake is adequate, rather higher energy intake could increase skin thickness (4, 16).

To the best of our knowledge, there have been no similar studies in Iran. Although research has been focused on the common use of nutrient-enriched formula in NICUs, the application and safety of milk supplements should be clarified. Poor growth during the NICU stay has long-term complications such as adverse neurodevelopment outcomes at 18–22 months corrected age. The present study aimed to evaluate the effect of protein supplements on the physical growth of ELBW infants.

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Methods
Study Design
This triple-blind, randomized clinical trial was conducted on the infants weighing less than 1,000 grams, who were admitted to the NICU of Akbar Abadi Hospital in Tehran, Iran during 2015-2016. In total, 64 infants were enrolled in the study and equally allocated to two groups of case and control. Sampling was initiated in September 2015, and the infants were followed-up until discharge from the hospital in February 2016.

Participants
All the infants weighing less than 1,000 grams were screened based on the inclusion criteria until the saturation of the sample size.

Inclusion criteria were as follows: 1) birth weight of less than 1,000 grams with the cessation of parenteral nutrition and milk intake of 100-120 mL/kg/day; 2) no major congenital disorders; 3) no diagnosis or suspicion of necrotizing enterocolitis (NEC) before using protein supplements; 4) no need for major surgeries; 5) no gestational infections restricting intrauterine growth and 6) no genetic disorders causing growth abnormalities.

Exclusion criteria of the study were increased blood urea nitrogen (BUN) to more than 25 mg/dL and symptoms of NEC and other clinical conditions requiring nil per os.

Randomization and Concealment
Using computer-generated block randomization with blocks of four infants, two infants were allocated to each group. The allocation was concealed until implementing the intervention in order to guarantee the quality of the study. After random allocations, both groups were matched in terms of weight, height, and head circumference in order to remove the effects of the confounding variables and form comparable groups for inferential analysis.

Growth factors were measured by a physician who was blinded to the study procedure; the study design was triple-blind (patient, researcher, and analyzer). All the infants were breastfed (100-120 ml/kg/day) within 2-3 hours. The case group received a protein supplement (0.6-0.8 g/kg/day) (Aptamil, Netherlands), which was added to their daily milk and set weekly. In the case of protein intolerance and vomiting, a lower dose of the supplement (0.6 g/kg/day) was administered. Total received protein was measured and recorded for each infant. Neonates in the case and control groups received breast milk fortifier (FMS, Aptamil, Netherlands) 4 -5 times per day as an in-between meal. Serum albumin was measured weekly as a positive factor to evaluate the amount of the protein supplement.

Monitoring of Adverse Effects
Blood samples were obtained weekly from the infants in order to measure BUN and creatinine (Cr), as well as the mean increase of NEC in the case and control groups. In the case of vomiting due to protein intolerance, we reduced the amount of protein in milk and increased it again after controlling the symptoms.

Enterocolitis
The classic symptoms of enterocolitis include abdominal dilatation, bloody stool, and pneumatosis

intestinalis. The other signs of the disease are unstable body temperature, lethargy, and other nonspecific findings (e.g., sepsis).

Outcome Measures

Primary outcome: To assess the growth rate of the infants, we measured their daily weight and weekly height and head circumference.

Secondary outcome: We recorded the incidence of morbidity risk factors, such as NEC, renal load increase, and increased BUN and Cr.

Sample Size Estimation

In the present study, the effect size was considered to be 0.7 based on similar articles (39). Sample size was calculated based on the following formula (40):

$$n \geq \frac{2(z_\alpha + z_\beta)^2 \sigma^2}{(\mu_1 - \mu_2)^2} = 2(1.96 + 1.28)^2 \left(\frac{1}{0.70}\right)^2 = 32$$

Where μ_1=1.4, μ_2=0.08, σ =1.62, $z_{1-\alpha/2}$=1.96, $z_{1-\beta}$ =1.28, and n=32.

In total, 64 neonates were selected and equally divided to the case and control groups.

Statistical Analysis

Data analysis was performed in SPSS version 24, and continuous variables were expressed as mean and standard deviation. Paired t-test was used to compare the means of continuous variables at the beginning and end of the study, and independent samples t-test was applied to survey the means of the two independent groups. In all the statistical analyses, the level of significance was 0.05.

Ethical Considerations

The study protocol was approved by the Ethics Committee of the Iranian Registry of Clinical Trials (ID: IRCT2016D13126115N2). This is a primary registry in the World Health Organization (WHO) Registry Network set up with the help of the Iranian Ministry of Health and Medical Education (MOHME) and hosted by Iran University of Medical Sciences (IUMS). Before the enrolment of the infants, informed consent was obtained from one parent of each subject.

Result

No significant differences were observed between the case and control groups in terms of the mean weight, height, and head circumference at birth and the beginning of the study. (Table 1). Considering the use of random sampling, no significant difference was observed in the gestational age between the case and control groups (P=0.004).

Table 2 shows the changes in the weight, height, and head circumference of the infants at the end of the study, as well as the differences in these indices at the beginning and end of the study between the groups. Mean daily weight gain in the case and control groups was 55.92±36.90 and 30.80±13.91 grams, respectively, and a significant difference was denoted between the groups in this regard (P=0.001).

Table 1. Characteristics of Infants

Variables	Mean ±SD		P-value
	Case (n=32)	Control (n=31)	
Gestational Age at Birth (week)	28.27±1.50	29.75±2.31	0.004
Birth Weight (g)	880.60±78.22	893.33±25.54	0.388
Height at Birth (cm)	36.59±1.89	36.62±1.78	0.949
Head Circumference (cm)	26.13±.86	26.16±1.17	0.908
Weigh at the Beginning of Study (g)	929.54±34.60	943.33±31.68	0.104
Height at the Beginning of Study (cm)	36.75±2.69	37.25±1.99	0.404
Head Circumference at the Beginning of Study (cm)	27.30±.87	27.40±1.48	0.746

Table 2. Changes in Growth Factors at the End of Study

Variables	Mean±SD		P-value
	Case (n=32)	Control (n=31)	
Weight at the End of Study (g)	1573.33±128.97	1456.66±117.95	<0.001
Height at the End of Study (cm)	39.49±1.95	39.24±2.29	0.642
Head Circumference at the End of Study (cm)	29.22±1.03	28.35±1.53	0.011
Mean Weight Difference at the Beginning and End of Study (g)	643.78±139.82	513.33±109.23	<0.001
Mean Height Difference at the Beginning and End of Study (cm)	2.73±2.33	1.98±0.50	0.084
Mean head Circumference Difference at the Beginning and End of Study (cm)	1.92±0.61	0.94±0.41	<0.001

Data analyzed by independent t-test

Table 3. Laboratory Data for Protein Tolerance

Variables	Case (n=32)		Control (n=31)	
	Mean±SD	P-value	Mean±SD	P-value
Albumin Level at the Beginning of Study (mg/dl)	3.70±0.36	0.005	3.37±0.38	0.001
Albumin Level at the End of Study	3.86±0.26		3.16±0.26	
BUN at the Beginning of Study (mg/dl)	10.18±1.92	0.651	10.78±1.79	0.03
BUN at the End of Study (mg/dl)	10.03±1.70		10.21±1.13	
Creatinine Level at the Beginning of Study (mg/dl)	0.70±0.093	<0.0001	0.75±0.13	<0.0001
Creatinine Level at the End of Study (mg/dl)	0.6212±0.04151		0.67±0.07	
Data analyzed by paired t-test				

No significant difference was observed between the case and control groups in terms of the mean weekly height growth, which was 0.77±0.67 and 0.76±0.29 centimeter in the case and control groups, respectively (P=0.939). However, a significant difference was denoted between the groups regarding the mean weekly head circumference growth, which was 0.51±0.1 and 0.34±0.16 centimeter in the case and control groups, respectively (P<0.001).

In the control group, one patient with NEC was excluded from the study, while no subjects were excluded from the case group. The amount of the protein added to milk was reduced from 0.8 to 0.6 g/kg/day in two patients in the case group due to protein intolerance. Moreover, serum albumin increased in the case group, while it decreased in the control group.

No significant increase was observed in the level of BUN in the case group (Table 3). In addition, the results showed reduced BUN in both groups, which could be attributed to the improvement of the glomerular filtration rate by increasing the age of the infants.

Discussion

This randomized, double-blind, clinical trial aimed to assess the effect of supplementary protein in daily milk on the growth of ELBW infants. The case and control groups were matched in terms of the related variables. Growth of premature and ELBW infants is an issue of great concern; for instance, improper growth of the head circumference after birth in premature infants is associated with deficient nervous system development and cerebral epilepsy (17). Therefore, physicians of premature infants should consider the key role of growth and weight gain in the survival of these newborns (18, 19).

Weight Gain in ELBW Infants

Several studies have indicated the effect of enriched nutrition on the weight gain and normal development of ELBW infants, proposing that a nutritional regimen enriched with adequate protein may result in weight gain regardless of the body fat content (20). Furthermore, another study in this regard has demonstrated that increasing the protein and energy in the daily intakes of neonates is associated with weight gain and increased Z scores, which in turn result in weight gain without gaining fat and lack of short-term clinical side-effects (21).

In a randomized, controlled, multi-clinical, open-label study, Procelli et al. (2000) evaluated the effect of milk-enriching agents (e.g., added protein) through comparison with the reference value on the premature infants weighing less than 1,000 grams. In the mentioned study, the growth and immune system parameters of the infants were measured weekly, and the case and control groups were matched in terms of demographic and baseline variables. According to the findings, the daily consumption of milk was significantly higher in the infants receiving the enriched formula compared to those fed with normal formula. Therefore, it was concluded that the new enriched formula could enhance the immune system of the neonates (similar to that of the reference milk), thereby increasing head circumference and causing faster weight gain than normal formula (22).

Findings of another study indicated that adding protein to the daily milk of premature infants increased their weight gain rate. Although the weight gain was observed in the control group, it was reported to be higher in the case group. In fact, infants receiving more protein showed a higher rate of weight gain compared to the control group, which indicated the pivotal role of protein in the daily nutrition of premature ELBW infants. On the other hand, head circumference of the premature infants in the mentioned research had a higher growth rate in the case group fed with added protein compared to the controls. Head circumference growth was observed in both groups, while it was significantly higher in the case group.

Weight gain and head circumference increase is of paramount importance in premature infants.

In fact, increased rate of weight gain may decrease the incidence of various diseases in premature infants, which in turn increases their life span. Use of breast milk fortified with protein supplements could increase the rate of weight gain, as well as height and head circumference growth, in preterm infants with favorable general health within a short interval. Moreover, these supplements could increase serum urea level, which indicates the adequacy of the applied regimen.

The mentioned study recommended further investigations to determine the required amount of protein supplement in daily meals for clinical improvement and long-term effects on the development of the nervous system in preterm ELBW infants (23); these findings are consistent with the results of the present study. Nervous system development was not considered in the current research due to the short study period, while it is being investigated in the ongoing studies on the same population. The results of these studies could be used in the prospective studies in this regard. Increasing the amount of protein in milk supplements could improve postpartum growth in hospitalized ELBW infants (24). The findings of these studies have also confirmed that using milk supplements could enhance the growth rate of premature infants, which is in line with the current research.

Amount of Protein Intake and the Complications

Various studies have been focused on the protein requirements of ELBW infants proposing contradictory results. In addition, the outcomes of adding protein to daily meals have been investigated in such studies. According to a research, the protein and energy added to the daily milk of premature infants within a certain interval exerted positive effects on their growth and development. The total amounts of 150 kcal/kg/day energy and 4.2 g/kg/day protein have been shown to be adequate to increase fat-free mass in premature infants (21).

On the other hand, a cohort has shown that higher amounts of protein than energy (based on the protein and energy amounts of 1.3 g/100 kcal and 8.2 g/100 kcal, respectively) is not associated with increased fat-free mass in ELBW infants; these findings have been confirmed by Fairey et al. (21, 25). In other words, taking more than 4.2 g/kg/day of protein may exceed the protein requirements of ELBW infants regardless of their energy intake. However, higher amounts of energy intake may alternatively improve protein catabolism. Therefore, more protein could be added to the energy-rich diets prescribed for these neonates (21).

A study by Sanchez et al. (2000) aimed to determine the proper concentrates to fortify breast milk, indicating that a concentrate containing protein, calcium, phosphorus, zinc salts, and vitamins A+D could be used to fortify human breast milk. In the mentioned study, the premature infants fed with 180-200 mL/kg/day of fortified breast milk showed better outcomes than the expected rate of growth and development as their nutritional requirements were met (26). In the current research, the nutritional requirements of the ELBW infants were met in the case group, and the subjects showed a more significant growth and development rate compared to the controls. However, different formulas were used to fortify breast milk in the present study similar to the available nutrient-enriched formula brands that use various formulas. Different brands of nutrient-enriched formulas could be tested on ELBW infants for the comparison of the results with the findings of the current research.

In the present study, the nutritional intake and weight gain of the ELBW infants were within the recommended limits although the protein intake was below the recommended range in the smaller infants (27). The infants in the current research were also breastfed and received 0.5-0.8 g/kg/day of protein. Mean daily weight gain in the infants was 55.92 g/day. Weight gain in the case group was higher than the control group, which indicated the effectiveness of the protein supplement.

The results of some studies are inconsistent with our findings, as well as the aforementioned studies. These studies have concluded that higher protein intake decreases growth impairment in breastfed infants, suggesting the use of higher levels of protein compared to the amount used in the present study (8). For instance, the results of a study on the safety of dietary supplements indicated that the safety of a new enriching concentrate was similar to that of previous concentrates, which promoted growth and development and prevented formula consumption in the infants admitted to the intensive care unit (ICU) (28). In the current research, a total of 0.6-0.8 g/kg/day of protein was administered to the subjects in the case group in addition to breast milk. NEC was not observed in the subjects, which indicated the safety of protein therapy in premature ELBW infants, as well as the appropriate amount of supplementary protein administered to the infants.

Proper weight gain has been reported to reduce the risk of sepsis, which could be justified by the higher mean weight gain in the case group in the present study. In addition, head circumference growth in the case group was significantly higher than the control group in our research, which predicted lower complications in the premature infants.

Height Growth and Neonatal Indices

Some studies have denoted that the patients with protein and energy deficiency, who are admitted to hospitals, do not relieve during hospitalization (7, 29-32). Moreover, previous studies have indicated that a high-energy diet without added protein could lead to impaired weight gain in human subjects (25). Although there have been biases in infant growth factors in assessing the effectiveness of nutrient-enriched formula and breast milk (33-36), using fortified breast milk has been shown to significantly reduce postpartum growth limitations in ELBW infants compared to those not receiving fortified milk (37). A study in this regard showed that infants fed with 75% breast milk were at a higher risk of poor growth and development, and the risk may even be higher in the infants who receive donated milk (38). Therefore, it could be inferred that rapid weight gain may reduce the risk of poor growth in infants. The results of the mentioned study are in congruence with the current research, indicating that weight gain and rapid growth in premature infants may reduce possible complications and outcomes.

Conclusion

According to the results, protein supplements (0.6-0.8 g/kg/day) added to daily milk could increase the mean weight gain and head circumference growth in premature ELBW infants. In addition, the mean weight gain and head circumference growth in such infants were higher compared to the newborns not receiving added protein.

A significant difference was observed between the case and control groups in terms of the mean weight and head circumference at the beginning and end of the study, while the difference in their height was not significant. In the case group (fed with protein supplements), the level of serum albumin was significantly higher than the controls although it was within the normal limit.

In the present study, there was only one case of NEC in the control group. However, two infants in the case group showed protein intolerance, and

the amount of added protein was reduced from 0.8 to 0.6 g/kg/day accordingly. Another treatment outcome was the increased BUN, which was not observed in the study subjects. Such poor outcomes have also been reported in the previous studies in this regard, indicating the safety of the treatments for the lack of weight gain in premature infants. Given the importance of weight gain in ELBW infants, this method is strongly recommended for preterm infants.

Since the subjects in the present study are being followed-up and undergoing two other studies on renal function and neurological development, the results of the current research could be helpful for these studies, as well as further similar research.

Acknowledgments

Conflicts of interests

None declared.

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