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Longitudinal Changes in the Macronutrient Contents of Breast Milk in the Mothers with Preterm Delivery

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ABSTRACT

***Background*:** Contents of breast milk may change during the first weeks after childbirth, especially in preterm deliveries. The present study aimed to determine the macronutrient contents of breast milk in the mothers with preterm delivery.

***Methods*:** This prospective-descriptive study was conducted in Mahdieh Hospital in Tehran, Iran in 2015. Participants included the mothers with preterm delivery, whose infants had a gestational age of <32 weeks and birth weight of <1500 grams. Samples of expressed breast milk were obtained from the mothers weekly for four weeks after delivery. Mid-infrared milk analyzer was used to measure the levels of the macronutrients in the samples. Exclusion criteria were mothers with chronic diseases, lack of access to the mothers in the first four weeks of delivery, and lack of consent to enroll in the study. Data analysis was performed in SPSS version 20 (SPSS Inc., Chicago, IL) and the quantitative variables were expressed in mean and standard deviation (SD). In addition, repeated measures ANOVA was used to evaluate the differences between the variables. P-value of less than 0.05 was considered statistically significant.

***Results*:** In total, 51 mothers were enrolled in the study. Mean gestational age and birth weight of the neonates were 28.44±2.20 weeks and 1064±260 grams, respectively. Mean age and body mass index (BMI) of the mothers were 27.5±5.6 years and 26.44±17.54-40 kg/m^2, respectively. In terms of socioeconomic status, 28 participants (60.8%) were categorized as class II (simple workers), and 20 mothers (45.4%) received basic prenatal care. During weeks 2-3 of delivery, the fat and calorie contents of breast milk increased significantly. However, protein content of breast milk was variable, and the carbohydrate level was observed to decrease. No significant associations were observed between the maternal age, socioeconomic status, BMI, and number of twins with the contents of breast milk, while the mode of delivery and level of prenatal care had significant effects on the protein level of breast milk.

***Conclusion*:** According to the results, the levels of macronutrients in the breast milk of the mothers with preterm delivery were variable and within the normal range during the first four weeks of delivery.

Keywords: Breast milk, Macronutrients, Preterm delivery, Preterm infant

Introduction

Breast milk of the biological mother is considered to be the optimal source of nutrition in term and preterm infants (1). Contents of breast milk vary in different lactating women and may change with each consumed meal depending on the nutritional contents of the mother's diet (2); for instance, fatty diets are likely to increase the lipid content of breast milk (3). Breast milk contents differ in the mothers with term and preterm delivery, longitudinal changes may occur within the first weeks of birth, especially in premature deliveries (4, 5).

Composition of the breast milk in the mothers with preterm delivery is compatible with the needs of the neonate. As determined by the 24-hour samples of breast milk collected during the first four weeks of lactation, the breast milk in the mothers with preterm delivery has higher protein and sodium concentration and lower calcium and phosphate

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contents compared to term deliveries (6). After the first days of life, breast milk cannot be the only source of nutrition for extremely preterm neonates; in such cases milk fortifiers could compensate for the deficiencies of the breast milk and prevent the osteopenia of prematurity as a complication of exclusive breastfeeding in premature infants (7).

Due to the changes in the contents of breast milk, it has recently been recommended that breast milk fortification must be individualized based on the needs of preterm newborns rather than following a preset schedule (8). Since breastfeeding is essential to the neurodevelopmental outcomes and prevention of necrotizing enteroculitis in premature neonates, measuring the macronutrient contents of breast milk could help evaluate the amount of nutrient intake in these infants. Furthermore, modification of the dietary plans in the mothers or individualizing the breast milk fortification plans could result in the proper growth of these neonates, thereby reducing the need for formula feeding.

Considering the rapid growth rate of preterm infants, the sufficiency of breast milk nutrients is as crucial to the optimal growth of these neonates as their intrauterine growth, while it could also eliminate the need for their formula feeding. However, the macronutrient composition of human milk varies in different individuals during lactation. In the mothers with term delivery, mean protein level is 0.91-2 g/dL, mean lipid level is 3.2-3.6 g/dL, and mean lactose content is 6.7-7.8 g/dL. The energy estimates are within the range of 65-70 kcal/dL, which are highly correlated with the lipid content of the human milk. As mentioned earlier, the macronutrient composition of breast milk differs in the women with term and preterm deliveries as the protein content tends to be higher than the lipid concentration in the mothers with premature delivery. In the women giving birth at weeks 32-33, the levels of protein, lipids, lactose, and calorie are estimated at 1.9 (1.3-2.5) g/dL, 4.8 (2.8-6.8) g/dL, 7.5 (6.5-8.5) g/dL, and 77 (64-89) kcal/dL, respectively (9).

Considering the changes in the macronutrient contents of breast milk, the present study aimed to determine the concentrations of lipids, calorie, carbohydrates, and protein in the breast milk of the mothers with preterm delivery in Mahdieh Hospital in Tehran, Iran.

Methods

This prospective- descriptive study was conducted at the NICU of Mahdieh Hospital affiliated to Shahid Beheshti University of Medical Sciences in Tehran, Iran in 2015. Mahdieh Hospital is a maternity medical center with 4,000-5,000 deliveries per year and 40 NICU beds. Mothers with preterm delivery (gestation: <32 weeks), whose neonates were admitted to the NICU with the birth weight of <1500 grams were enrolled in the study. The study protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, and written informed consent was obtained from all the participants.

Exclusion criteria of the study were chronic maternal diseases, lack of access to the mothers during the first four weeks of delivery to provide breast milk, and lack of consent for participation in the research. Samples of expressed breast milk were obtained from the participants weekly, since the end of the first week to the fourth week of delivery.

Mid-infrared milk analyzer (MIRIS analyzer, Sweden) was used to measure the concentration of macronutrients in the breast milk samples through mid-infrared transmission spectroscopy. The MIRIS analyzer measures the levels of total protein and true protein (true protein measurement crude protein deduced by non-protein-nitrogen [NPN]), carbohydrate [lactose and oligosaccharides] solids, lipids and calories. In accordance with the instructions of the manufacturer, five milliliters of fresh expressed non-diluted breast milk without any chemical additives was mixed with the samples in order to prevent the separation of the fats and other contents of the breast milk. Afterwards, the samples were indirectly heated to 40°C and analyzed in terms of the macronutrients using breast milk analyzers.

Sampling was performed at 2-4 PM on all the mothers with a similar duration. The macronutrient contents of the breast milk samples were measured and compared within four weeks after the preterm deliveries. Moreover, we investigated the effects of variables such as the mode of delivery, socioeconomic status, prenatal care, maternal age, maternal BMI, gestational age, birth weight, and number of twins on the macronutrient contents of breast milk. It should be noted that the participants were classified into four categories in terms of the socioeconomic status, including unemployed, simple workers, skilled workers and public servants, and privileged.

Prenatal cares (PNC) were also classified into

four levels of no PNC, basic PNC (every four weeks until week 28 of gestation in uncomplicated pregnancies, every two weeks in weeks 28-36 of gestation, and weekly until delivery), advanced PNC (more frequent than the routine schedule in high-risk pregnancies), and highly advanced PNC (requiring the supervision of perinatologists or admission in perinatology wards).

The participants were classified into four categories in terms of maternal BMI, including underweight (<18.5 kg/m2), normal (18.5-24.9 kg/m2), overweight (25-29.9 kg/m2), and obese (>30 kg/m2) in accordance with the classification guidelines of the World Health Organization (WHO).

In the mothers who stayed in the hospital for neonatal care, hospital food was considered as the main source of nutrition during the study. There were no special diets in the mothers, while we considered the differences in the dietary plans of the mothers who stayed at home during the study.

Data analysis was performed in SPSS version 20 (SPSS Inc., Chicago, IL), and the quantitative variables were expressed in mean and standard deviation (SD). In addition, repeated measures ANOVA was used to evaluate the differences in the macronutrient concentrations of the breast milk samples. In all the statistical analyses, P-value of less than 0.05 was considered significant.

Results

In total, 51 mothers with preterm delivery were enrolled in the study. Mean gestational age was 28.44±2.20 weeks (range: 25-32 weeks), mean birth weight of the neonates was 1064±260 grams (range: 670-1700 grams), and mean maternal age was 27.5±5.6 years (range: 17-45 years). In 14 cases (28%), the mode of delivery was natural vaginal delivery (NVD), while it was cesarean section (C/S) in 36 cases (72%).

Mean BMI of the mothers was 26.44 kg/m^2 (range: 17.54-40 kg/m^2). In this regard, two participants (5.2%) were underweight, 16 cases (42.1%) were normal, 13 cases (34.2%) were overweight, and seven mothers (18.4%) were obese with regard to the socioeconomic status, five participants (10.8%) were unemployed,28 cases (60.8%) were simple workers, six cases (13%) were skilled workers and public servants, and seven participants (15.2%) belonged to the privileged class. Among the participants, one mother (2.2%) received no PNC, while 20 cases (45.4%) received basic PNC, 17 cases (38.6%) had advanced PNC, and six participants (13.6%) were provided with highly advanced PNC.

Mean levels of calorie, total protein, true protein, and lipids and solids in the breast milk samples were 66.9 kcal/lit (range: 44-95 kcal/lit), 1.92 g/dL (range: 1.1-4 g/dL), 1.57 g/dL (range: 0.8-3.6 g/dL) 4.38 g/dL (range: 1.2-7.5 g/dL), 6.02 g/dL (range: 1.2-16 g/dL) and 12.71 g/dL (range: 9.6-16 g/dL), respectively. Mean levels of the macronutrients and calorie during the first four weeks of delivery are presented in Table 1.

Diagrams 1-6 depict the patterns of the changes in the breast milk macronutrients during the study period (four weeks, accordingly, the lipid and calorie levels in the breast milk samples increased significantly within the weeks 2-3 of delivery. Despite the gradual reduction in these macronutrients, their concentrations were higher compared to the first week after delivery. In contrast, the level of carbohydrates remained stable during the weeks 1-3 of delivery, followed by a significant reduction after three weeks.

The protein contents (true and total) of the breast milk samples were variable and decreased every week since the first week until the fourth week after delivery. The pattern of the changes in the solid contents of the breast milk samples was similar to that of the lipids and calories. According to our findings, the longitudinal changes in the levels of calorie (P<0.001), solids (P=0.02), and true and total protein (P=0.001 and P<0.001, respectively) had significant differences within the four weeks after delivery.

Table 1. Mean Levels of Macronutrients in Breast Milk of Mothers with Preterm Delivery

	Week 1	Week 2	Week 3	Week 4	P-value
Calorie (kcal/lit)	67.5±1.87	54±5.47	74.4±1.28	71.1±.71	0.001*
Carbohydrate (g/dL)	4.46±.27	4.47±.39	4.5±.19	4.07±.15	0.557
Solids (g/dL)	12.6±.24	11.9±.49	13.2±.20	13±.14	0.022*
Lipids (g/dL)	5.77±.24	5.56±.41	6.35±.21	6.38±.14	0.053
True Protein (g/dL)	1.65±.11	1.40±.06	1.83±.09	1.40±.04	0.001*
Total Protein (g/dL)	2.09±.11	1.68±.09	2.16±.09	1.73±.04	<0.001*

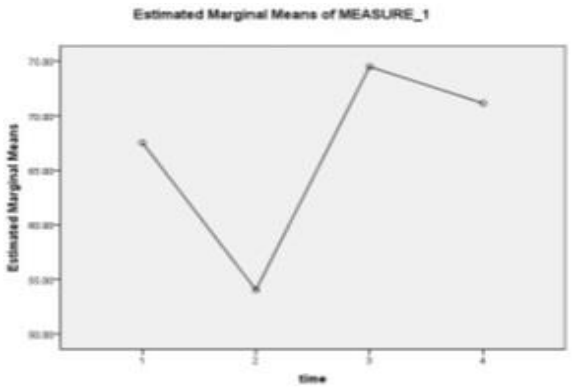

Diagram 1. Changes in Calorie Content of Breast Milk

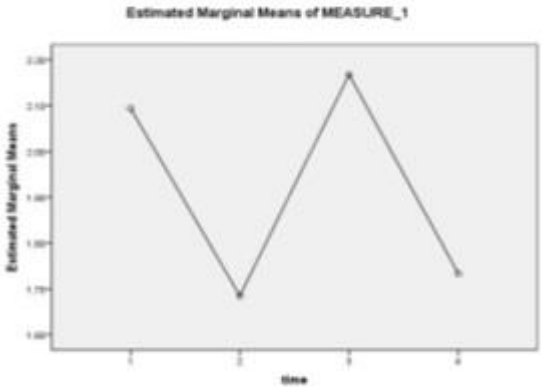

Diagram 2. Changes in Carbohydrate Content of Breast Milk

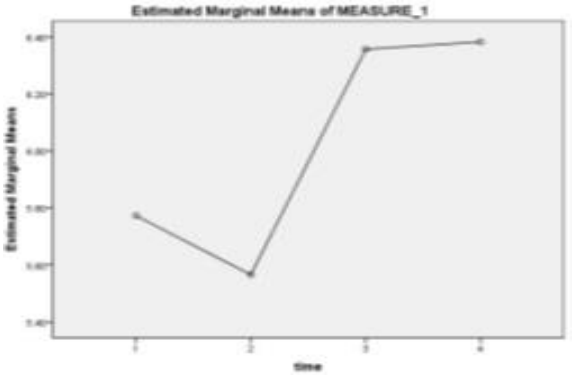

Diagram 3. Changes in Electrolytes of Breast Milk

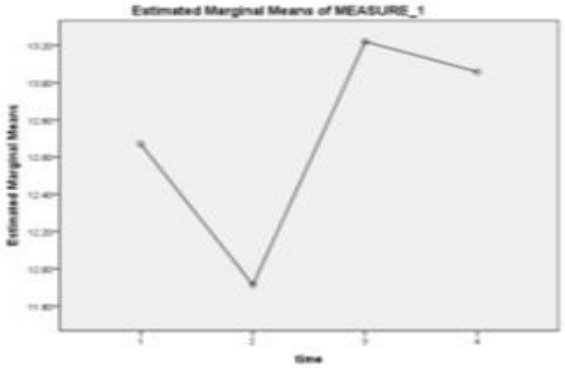

Diagram 4. Changes in Lipid Content of Breast Milk

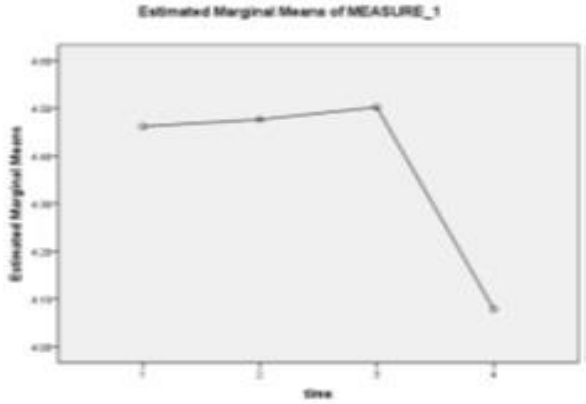

Diagram 5. Changes in True Protein Content of Breast Milk

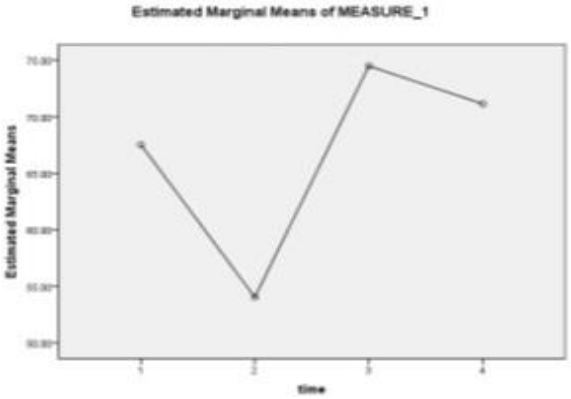

Diagram 6. Changes in Total Protein Content of Breast Milk

The correlations between the mode of delivery, socioeconomic status, PNC, maternal age, maternal BMI, gestational age, birth weight, and multiple pregnancies with the macronutrient contents of breast milk are shown in Table 2. Since the current research was conducted in a referral tertiary center, the rate of multiple pregnancies was relatively high.

According to the results of the present study, there were no statistically significant differences between the maternal age, socioeconomic status, maternal BMI, and the number of twins with the contents of all the macronutrients in breast milk. However, the mode of delivery (NVD or C/S) ($P<0.001$), PNC ($P<0.001$), and birth weight ($P=0.04$) had significant effects on the true and total protein contents of breast milk ($P=0.033$ and $P=0.026$, respectively). Furthermore, gestational age of the neonates showed a significant difference in terms of the lipid content ($P<0.016$) and total protein content of breast milk ($P<0.032$).

Table 2. Correlations of Variables and Macronutrient Contents of Breast Milk

	Calorie (kcal/lit)	Carbohydrate (g/dL)	Solids (g/dL)	Lipids (g/dL)	True Protein (g/dL)	Total Protein (g/dL)
Mode of Delivery						
NVD (n=14)	70.2±2.9	4.54±0.3	13±0.3	5.78±0.35	1.63±0.08	2±0.96
C/S (n=36)	65.4±1.8	4.31±0.18	12.6±0.192	6.11±0.218	1.55±0.05	1.88±0.6
Mode of Delivery (P-value)	0.182	0.522	0.262	0.434	<0.001*	<0.001*
Maternal Body Mass Index (BMI)						
Underweight (n=2)	73.53±6.5	3.840 ±0.71	12.65±0.62	6.205 ±0.95	1.925±0.23	2.550 ±0.23
Normal (n=16)	71.34±2.3	4.718 ±0.25	13.14±0.22	6.229 ±0.33	1.533±0.81	1.846 ±0.08
Overweight (n=13)	63.57±2.5	3.947±0.27	12.36±0.24	5.694 ±0.37	1.531±o.90	1.829 ±0.09
Obese(n=7)	67.54±3.5	4.527±0.38	13.125±0.33	5.692±0.51	1.683±0.12	2.076±0.12
BMI (P-value)	0.146	0.196	0.115	0.691	0.326	0.24
Socioeconomic Status						
Unemployed (n=5)	66.83±4.7	4.16 ±0.51	12.53±0.49	6.61±0.61	1.66±0.14	2.14±0.16
Simple Worker (n=28)	64.4±2	4.43 ±0.21	12.49±0.2	5.96±0.25	1.52±0.59	1.92±0.70
Skilled Worker/Public Servant (n=6)	75.3±4.3	4.74±0.46	13.71±0.45	5.43±0.55	1.75±128	1.81±0.15
Privileged (n=7)	70.95±4	4.20±0.43	12.94±0.41	6.10±0.51	1.53±0.118	1.83±0.14
Socioeconomic Status (P-value)	0.115	0.804	0.108	0.556	0.391	0.453
Prenatal Care						
None (n=1)	71.9±10.2	3.86±1.1	12.40±0.98	6.86±1.37	1.91±0.22	3.05±0.32
Basic (n=20)	69.18±2.3	4.55±0.25	12.75±0.22	6.20±0.3	1.619±0.12	1.82±0.07
Advanced (n=17)	69.41±2.4	4.39 ±0.27	13.04±0.23	5.82±0.33	2.284±0.17	1.89±0.07
Highly Advanced (n=6)	63.86±4.2	4.75±0.46	13.10±0.40	5.97±0.56	1.520±0.88	2.09±0.13
Prenatal Care	0.675	.841	.727	.781	.001*	.004*
Multiple Gestations						
Single (n=22)	66.10±2.4	4.69±0.24	12.66±0.25	5.57±0.27	1.547±0.06	2±0.79
Twins (n=10)	64.78±3.6	4.20±0.36	12.54±0.37	5.8±80.41	1.756±0.09	1.85 ±0.11
Triplets (n=11)	72.22±3.4	4.33±0.34	13.07±0.35	6.84±0.39	1.431±0.09	1.76±0.11
Quadruplets (n=1)	70.8±1.1	3.51±1.1	13.31±1.1	6.43±1.2	.592±0.3	2.17±0.37
Multiple Gestations (P-value)	.434	.536	.699	.079	.121	.297
Birth Weight	.911	.350	.232	.097	.033	.026
Maternal Age	.566	.987	.690	.103	.352	.757
Gestational Age	.169	.355	.309	.016	.704	.032

Discussion

The present study was a prospective research conducted on the mothers with preterm delivery in Mahdieh Hospital in Tehran, Iran in order to measure the macronutrient contents in breast milk, as well as their longitudinal changes within the first four weeks after delivery. According to the findings, 42.1% of the participants had normal BMI (18.5-24.9 kg/m^2), while 34.2% were overweight (25-29.9 kg/m^2). In addition, the number of the mothers with under nutrition and obesity was significantly lower than the mentioned subjects. A research in this regard was performed by Anjana Verma and Lalit Shrimali (2012) in India (10) on 784 pregnant women, 116 of whom were underweight (14.79%), and 406 cases were normal (51.78%), while 165 (21.04%) and 84 subjects (10.71%) were overweight and obese, respectively.

Although 60.8% of the participants were simple workers in the present study, only one case (2.2%) had no PNC, while 45.4% received basic PNC based on the routine protocol, and 38.5% had advanced PNC. Similarly, Ping Ling Yeoh (2016) conducted a research in Malaysia (11) regarding antenatal care (ANC) utilization, in which 63% of the women (n=330/522) had adequate-plus or intensive ANC utilization, while 21% (n=107/522) had inadequate ANC utilization. The mentioned research showed the disproportionate utilization of ANC based on the risk of the assessed pregnancies, highlighting the need for improving the care schedule. As discussed earlier, the macronutrient composition of human milk varies in different women during lactation. In the present study, variations were observed in the levels of the macronutrients in the breast milk samples. Accordingly, the levels of lipids and calories changed with a similar pattern, decreasing in the second week of delivery, followed by a significant increase. Considering that lipids and calories are conspicuous sources of energy in breast milk, the similarity in the change pattern of these macronutrients is justified.

In a study by Gitte Zachariassen (2013) (12), the changes in the lipid and calorie levels were similar to the current research. According to the

mentioned study, the mean level of lipids and lactose remained stable in the entire period of the study, while we denoted the higher level of carbohydrates in the first two weeks of delivery, as well as the third and fourth weeks. Meanwhile, the level of lipids was observed to increase in this period. Differences in the change patterns of the macronutrients could be due to the duration of the mentioned study since the study by Gitte Zachariassen was performed since the second week until six months after delivery, while our research was carried out during the first month of delivery.

According to the results of the present study, changes in the concentrations of calories, solid nutrients, and proteins had statistically significant differences during the first four weeks after delivery, the trend of changes in the protein levels was variable, without a regular pattern of increase or reduction. This could be due to the variable protein intake in the diets of the studied mothers. In the study by Gitte Zachariassen, the protein content of human milk was reported to vary noticeably in different mothers, decreasing within weeks after extremely preterm birth.

Similar to the current research, the study by Aiko Menjo (2009) (13) indicated that the lipid and protein contents of breast milk significantly variable within and among the mothers with very-low-birth-weight infants (birth weight: <1500 grams).

Comparison of the mean macronutrient levels between the present study and findings of Olivia Ballard (2013) (9) demonstrated that the calorie and protein levels were similar, while the level of carbohydrates was lower in our study. On the other hand, the level of lipids was higher in the current research compared to the mentioned study. This discrepancy could be due to the differences in the time of sampling the breast milk or maternal diets.

Comparison of term and preterm deliveries in terms of the macronutrients contents in breast milk in the current research indicated that the level of calories was similar, while in preterm pregnancies, the carbohydrate level was lower, lipid level was higher, and total protein level was slightly higher than term pregnancies (12). Some findings in this regard have shown that the lipid content of the maternal diet could affect in the lipid level such as the study by Nikniaz L (2009) in Iran (3). However, we did not evaluate the effect of maternal dietary lipid content on the level of lipid in breast milk.

In a research conducted by Enrico Bertino (2012) (14), breast milk of the mothers with term and preterm pregnancies was compared, indicating that lower lactose concentration could positively affect the nutrition of preterm neonates since lactose contributes to lower milk osmolality. Moreover, low lactose levels might represent the lower substrate levels of common lactase deficiency, which often occurs in preterm neonates. In the present study, the level of carbohydrates was observed to be low in the breast milk of the mothers with preterm delivery although MIRIS analyzer measured the total carbohydrate (lactose and oligosaccharides).

In the current research, multivariable analysis demonstrated that in the mothers with preterm delivery, there was a statistically significant difference between the mode of delivery and protein contents of the breast milk ($P<0.001$). In a study conducted by Evrim Alyamac Dizdar (2014) (15) regarding the effects of the mode of delivery on the macronutrient contents of breast milk, similar results to the present study were reported. In the mentioned research, the authors concluded that NVD is associated with higher colostrum protein content. In addition, labor pain and uterine contractions induce hormonal activities and alterations in the protein composition of the human milk in order to facilitate the optimal development of important physiological functions in the newborn.

In the present study, maternal BMI had no effects on the macronutrient contents of breast milk. However, the results obtained by Yang Titi (2014) in China (16) denoted that maternal BMI was positively associated with lipid content of breast milk, and the effect was greater than the dietary intake of fats. In the mentioned research, all the other maternal characteristics had no significant effects on the breast milk composition. The study by Yang Titi performed on the mothers with term delivery, while our participants were women with premature delivery. Differences in the gestational age could explain the inconsistency regarding the effect of BMI on the lipid content of the breast milk.

According to the information in Table 2, the level of PNC had variably significant effects on the protein content of breast milk. With regard to the total protein content, the group receiving highly advanced PNC had higher protein levels compared to the others, while the true protein level was observed to be lower in the mothers receiving highly advanced PNC compared to the other subjects. This could be due to the fact that women with high-risk pregnancies require

more advanced PNC, and their underlying diseases may also affect the protein contents of their breast milk.

In the current research, socioeconomic status of the mothers had no significant effects on the macronutrient contents of breast milk. Considering that only five participants (10.8%) in our study had a low socioeconomic level, this finding could be attributed to the small sample size of this group as a confounding variable.

Variability in the macronutrient composition of expressed human breast milk may be due to the inadequate nutrient intake of the preterm infants in terms of their estimated needs. Recent findings emphasize on the individualized fortification of breast milk in preterm infants based on their nutritional needs, which necessitates the macronutrient analysis of breast milk. In a study performed by Virginie de Halleux (17) regarding the benefits of individualized fortification, the authors concluded that the variability in breast milk composition was high in terms of the energy and protein contents, and this variability persists after standard fortification. Therefore, individualized breast milk fortification could optimize the protein and energy intake. However, we did not investigate the effects of breast milk fortification in this regard.

One of the limitations of the present study was the measurement of breast milk contents within only four weeks after delivery since the results might have differed in the cases if the study had continued. Small sample size was another limitation of our research. Comparison of the neonatal growth markers with breast milk contents may be a more practical approach to evaluate the sufficiency of breast milk for the normal growth of preterm infants.

Conclusion

According to the results, the contents of macronutrients in breast milk are not consistent and may vary longitudinally. Moreover, no significant correlations were observed between maternal age, socioeconomic status, and BMI with breast milk contents. A positive association was denoted between NVD and the protein contents of breast milk, as well as the, other benefits of NVD, in terms of nutrition, which could be more appropriate to improve the growth of preterm infants. This issue emphasizes on the selection of NVD for delivery of preterm infants. The current research showed that the mothers who received more PNC had lower levels of true protein, which is an essential macronutrient for the growth of neonates. It is possible that mothers with complicated pregnancies must pay special attention to efficient diets in order to increase the protein concentration in their breast milk.

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