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The Role of Infant Sex on Human Milk Composition

Mohammadbagher Hosseini,[1] Einollah Valizadeh,[2] Nafiseh Hosseini,[3] Shirin Khatibshahidi,[4] and Sina Raeisi[1]

Abstract

Objective: Human milk (HM) is the most necessary and complete food for infants and their survival. It is a dynamic system influenced by different factors such as the sex of the infant. The study of the factors affecting the composition of mother's milk can provide us better insight into the nutritional needs of infants with different sex and improve the nutrition of babies who do not have access to HM. This study aimed to study the effects of infant sex on mother's milk composition.

Methods: Sixty-one mother's milk samples were collected from mothers with female or male infants and divided into two (32 sons, 29 daughters) groups. The samples were analyzed by the Lacto-Scan milk analyzer and the obtained data were compared between the groups.

Results: The results showed that the mean fat content in the milk samples of mothers with female infants (daughters group) ($3.42 \pm 1.80\%$) was significantly ($p = 0.029$) higher than that in mothers with male infants (sons group) ($2.53 \pm 1.18\%$). However, the sons group had higher ($p = 0.024$) levels of salts ($0.76 \pm 0.14\%$) compared with the daughters group ($0.67 \pm 0.18\%$). There was no significant ($p > 0.05$) difference in other indices between the groups.

Conclusions: The sex of the infant is one of the important factors affecting the composition of mother's milk. The difference in the composition of mother's milk may reflect the differences in metabolic substrate needed for optimal growth and development in female and male infants.

Keywords: human milk, infant sex, milk composition

Introduction

The preponderance of breastfeeding has been fully marked over the years.[1] Human milk (HM) has been deemed as the gold standard for infant nutrition after birth.[2] This valuable source contains all the necessary nutrients including carbohydrates, proteins, fats, and salts required for healthy growth and development of infants.[2,3] The biochemical composition of HM is profoundly dynamic and can be altered during the lactation period.[3] Moreover, it can be affected by maternal nutritional status, genetic diversity, innate characteristics, and fetal cues.[4]

According to previous data, changes in HM composition not only is dependent upon lactation stage but also it is sex specific.[2] Infants of different sexes may have different and specific nutritional demands for optimum growth and development.[3] Male infants are more susceptible to complications than female infants regarding the lower Apgar scores, higher need for supplemental oxygen, higher rates of respiratory illnesses, and higher incidences of perinatal mortality.[3,5] Of interest, mother's milk may be specifically tailored for each sex; hence, mothers may produce HM with different composition for each male and female infant.[6] Simply put the tailoring of early life nutrition can be assumed as an essential mechanism for maintaining health, improving growth and development, and protecting against probable complications in both male and female infants.[3,5,6] Several animal model studies have shown that mothers produce different milk composition for male and female offspring.[7–12] Hinde et al.[7] in a bovine model study showed that mothers produced significantly higher milk volume and energy content for the female offspring. In studies[8,9] conducted on monkeys (*Macaca mulatta*), it was reported that the milk of mothers with male offspring had higher energy but lower volume and calcium levels than the milk of mothers with female infants.

[1]Pediatric Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[2]Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[3]Department of Pharmacology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.
[4]Alzahra Teaching Hospital, Tabriz University of Medical Sciences, Tabriz, Iran.

Although experimental models[7–12] have demonstrated that animal milk composition differs according to offspring sex, the present data for the sex specificity in HM composition are limited and conflicting.[4,13,14] In addition, there has been limited analysis of HM compounds including carbohydrates, fats, proteins, and salts. Therefore, this study intended to evaluate the main biochemical factors of the HM affecting health and growth including protein, fat, lactose, salts, and pH, and compare them for the first time between Iranian mothers with male or female infants. There are, at present, no practice guidelines to set different nutritional plans or formulas for male and female infants.[15] Thus, a precise comprehension of whether male or female infants drink HM with different compositions can be valuable enough to tailor proper nutritional plans in a sex-specific feature.

Materials and Methods

Design

This comparative cross-sectional study was carried out among 119 Iranian lactating mothers of male or female infants who were volunteers referring to Human Breast Milk bank of Al-Zahra Hospital of Tabriz University of Medical Sciences for donating their HM between September 2017 and September 2018. It is the first HM bank in Iran that was established in 2015. It has been developed based on the standards and guidelines presented by the HM Banking Association of North America.[16]

Sample collection and analysis

Only 61 of 119 healthy mothers who were exclusively breastfeeding, had successfully passed the routine pregnancy screening tests, and whose infants were 10 days to 2 months old with complete data were involved in the study. This period was chosen because the composition of HM during the first 10 days after birth (Stage III Lactogenesis) may be different from mature milk. The composition of HM then changes slowly over the months of full exclusive breastfeeding.[17] The exclusion criteria were smoking and drinking more than two ounces of alcohol per day. Mothers whose infants were preterm (<37 weeks of gestation) and had any major disorders or congenital malformations were excluded from the study. Mothers with twin or triplet infants and vegans with no vitamin B12 supplementation in their diet were also excluded from the study. The study flowchart is given in Figure 1.

The mothers were invited by the hospital to come to the HM bank. Informed consent was obtained from all mothers after birth before including in the study. Mothers were divided into ''Sons'' ($n=32$) and ''Daughters'' ($n=29$) groups according to the sex of their infants. Each mother manually expressed 10 mL of the milk as a sample between 6 and 10 am after the infant had been fed for 3 minutes.[18] The samples were immediately aliquoted in sterile test tubes and frozen at −70°C until the analysis. Then, the samples were analyzed for fat, solids-not-fat (SNF), density, lactose, salts, protein, and pH by an ultrasonic milk analyzer (Lactoscan MCC; Milkotronic Company, Nova Zagora, Bulgaria), which had been previously calibrated by MIRIS HM Analyser (Miris AB, Sweden). The study protocol has been approved by the institutional review board.

Data collection

A comprehensive set of questionnaires were developed and were applied to gain information on demographic characteristics of the mothers [age, body mass index (BMI), and parity] and infants (age, birth weight, birth length, head circumference, and gestational age) by the research team including physicians and nurses at the time of sample collection. The BMI was calculated by measuring the weight and height of mothers at the sample collection time. The data of milk composition (fat, SNF, density, lactose, salts, protein, and pH) were added to the questionnaires by the research team after the HM analysis.

Data analysis

The data were analyzed by SPSS software (version 16.0; SPSS, Inc., Chicago, IL). Initially, the variables were statistically checked for normality by one-sample Kolmogorov–Smirnov test. All variables except pH had normal distributions and therefore were shown as mean ± standard deviation and compared by independent samples *t*-test. The pH was shown as median with interquartile range and compared by Mann–Whitney *U*-test. The correlation was analyzed by Pearson's correlation coefficient method. A value of $p<0.05$ was considered statistically significant.

Results

The characteristics of the mothers and infants are given in Table 1. As given in the table, there was no significant difference in maternal characteristics including age, BMI, and parity between the studied groups ($p>0.05$). Age, birth weight, birth length, head circumference, and gestational age as neonatal characteristics of the infants were not either significantly different between the groups ($p>0.05$).

Table 2 provides the evaluated factors of the mother's milk samples between the studied groups. The results showed that the mean HM fat content in the daughters group (3.42 ± 1.80%) was significantly ($p=0.029$) higher than that in the sons group (2.53 ± 1.18%). Nevertheless, the sons group had higher ($p=0.024$) levels of salts (0.76 ± 0.14%) compared with the daughters group (0.67 ± 0.18%). There was no significant difference in other evaluated indices including SNF, density, lactose, protein, and pH between the groups ($p>0.05$). There were significant negative correlations between the evaluated indices, salts, and fat in both sons ($r=-0.668$, $p<0.001$) and daughters ($r=-0.949$, $p<0.001$) groups (Fig. 2a, b). Any other significant correlation was not found among the evaluated factors.

Discussion

Male and female infants have different growth and developmental responses to environmental stimuli.[3] Maternal sex-specific milk synthesis and early life nutritional care as well may be necessary to maintain both sexes healthy and protect them against complications that may be faced early in life.[11,13,14]

Robert and Braun[10] in a study on tammar wallabies (*Macropus eugenii*) revealed that mothers of male offspring, unlike that of the female offspring, produced milk with a higher protein level. The milk volume and energy content were not different for both sexes. Quesnel et al.[11] in the other

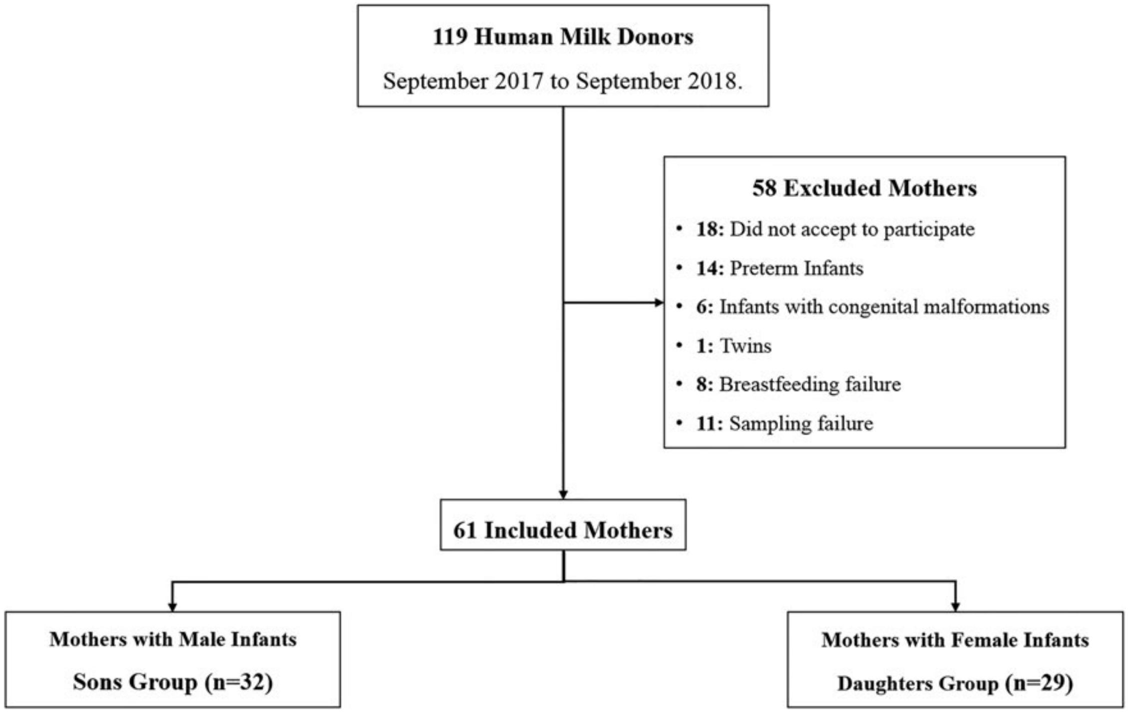

FIG. 1. The study flowchart. There were 119 lactating mothers who were volunteers referring to Human Breast Milk bank of Al-Zahra Hospital of Tabriz University of Medical Sciences, Tabriz, Iran, for donating their HM between September 2017 and September 2018. Fifty-eight mothers were excluded from the study: 18 mothers declined consent for the study, 14 mothers had preterm infants, 6 mothers had infants with congenital malformations, and 1 mother had twins. Eight and 11 cases were also excluded from the study because of breastfeeding and sampling failures, respectively. Eventually, 61 mothers were included in the study and divided into two ''Sons'' ($n = 32$) and ''Daughters'' ($n = 29$) groups according to the sex of their infants. HM, human milk.

study on wild eastern kangaroos (*Macropus giganteus*) reported similar results. Iberian red deer (*Cervus elaphus hispanicus*) mothers were found in a study by Landete-Castillejos et al.[12] to produce more volume of milk with higher levels of fat, lactose, and protein for male offspring.

The association of infant sex with HM composition has also been evaluated by some clinical trials.[4,13,14,18–20] However, the available results were conflicting. For instance, Powe et al. showed that American mothers produce milk with a higher energy content for male infants,[4] whereas, according to Fujita et al.[13] and Hahn et al.,[14] Kenyan and Korean mothers' milk contained higher energy content for female infants. Of interest, Fujita et al.[13] also showed that only mothers with low socioeconomic status produced milk for their female infants with a higher fat concentration. Altufaily[19] reported that Iraqi mothers of female infants produced milk with a higher calcium concentration, however, the lower volume and phosphorus content compared with the mothers of male infants. Conflicting with these results, no connection was detected by Quinn.[18] between the milk composition of Filipino mothers and infant sex. These studies had several limitations

Table 1. Demographic Characteristics of the Mothers and Infants

Maternal characteristics	*Mothers/daughters*	*Mothers/sons*	p-*value*
Age (years)	23.16 ± 5.49	25.65 ± 6.33	0.255
BMI (kg/m^2)	28.13 ± 5.48	27.80 ± 5.00	0.180
Parity	2.41 ± 1.13	2.75 ± 1.44	0.111
Neonatal characteristics			
Age (days)	29.13 ± 7.70	27.55 ± 7.10	0.230
Birth weight (g)	3615.10 ± 544.30	3510.70 ± 540.40	0.240
Birth length (cm)	52.12 ± 2.35	50.40 ± 2.65	0.130
Head circumference (cm)	36.31 ± 1.50	35.50 ± 1.50	0.055
Gestational age (weeks)	39.50 ± 1.90	39.8 ± 1.70	0.710

Data are given as mean ± standard deviation and were compared using independent samples *t*-test.
BMI, body mass index.

TABLE 2. BIOCHEMICAL FACTORS OF THE BREAST MILK SAMPLES BETWEEN THE STUDIED GROUPS

Factor	*Sons*	*Daughters*	p-*value*
Fat (%)	2.53 ± 1.18	3.42 ± 1.80	0.029[a,*]
SNF (%)	9.00 ± 0.90	8.86 ± 0.44	0.430[a]
Density	30.20 ± 2.94	30.07 ± 1.41	0.816[a]
Lactose (%)	7.06 ± 0.69	7.04 ± 0.34	0.853[a]
Salts (%)	0.76 ± 0.14	0.67 ± 0.18	0.024[a,*]
Protein (%)	1.14 ± 0.11	1.14 ± 0.05	0.865[a]
pH	8.10 (7.98–8.15)	8.13 (8.09–8.16)	0.102[b]

[a]Data are given as mean ± standard deviation and were compared using independent samples *t*-test.
[b]Data are given as median with interquartile range and were compared using Mann–Whitney *U*-test
*Statistically significant ($p < 0.05$).
SNF, solids-not-fat.

and were conducted in different conditions and with various analytical processes explaining the dissimilarities in the reported results.

In this study, it was found for the first time that Iranian mothers of female infants produced milk with higher fat but lower salt content compared with mothers of male infants. Salt is a vital nutrient for all cells. It may affect child growth and development. It has been revealed that salt restriction in gestation period could lead to retardation of fetal growth, consequently causing low birth weight and survival rates.[21] Chou et al.[22] showed that the birth and survival rates among pups of salt-sensitive rats with low salt intake were lower than pups of rats consuming a higher salt diet during mating and gestation. Salt may also be crucial for the development of the nervous system. It was revealed by Bursey and Watson[23] that salt restriction caused retardation of brain development in Sprague-Dawley rats. Low salt intake can lead to upregulation of the renin–angiotensin system.[24] Enhanced renin–angiotensin system activity may promote cardiovascular and renal injury mechanisms.[21,25] The other low salt intake causing alterations in metabolism may be related to epigenetic mechanisms and a decrease in insulin sensitivity.[21] Overall, salt intake may have a crucial role in growth and survival of infant not only by the mother in the gestational period, but also after birth by infant during breastfeeding. As mentioned, male infants are more sensitive and susceptible to complications than female infants. Higher levels of salt in HM for male infants may be a compensatory mechanism for maintaining their health, growth, and development, and protecting them against probable complications.

Sex-related differences in body fat mass have been shown to be present even during the first months of life.[26] Previous studies[26–28] revealed that female offspring have higher fat mass and less fat-free mass than male infants not only in newborns, but also among children and adolescents. Female infants can use fat stores as energy reserve or against heat loss in the first days after birth, resulting in better outcomes seen in female neonates compared with the male.[26] Therefore, female infants with higher whole-body fat mass may need to intake HM with higher fat content.

Although the mechanisms of underlying sex-specific milk synthesis have not yet been impeccably clarified, some mechanisms and factors might be of great prominence. It has been reported that the maternal mammary glands may be affected prenatally by fetal factors that are different in male and female fetuses.[7] Fetal steroid hormones have been detected from the first trimester of gestation in lactating dairy cows.[29] Bovine insulin-like peptide 3 (INSL3), another fetal-origin factor was detected by Anand-Ivell et al.[30] in maternal circulation during pregnancy. In addition, they found that this factor was higher in the blood of the cows with a male baby than the cows with a female baby.

Milk produced by mothers with high BMI was found in a study by Fields et al.[20] to contain higher leptin and insulin levels for female infants. Placental lactogen, a hormone

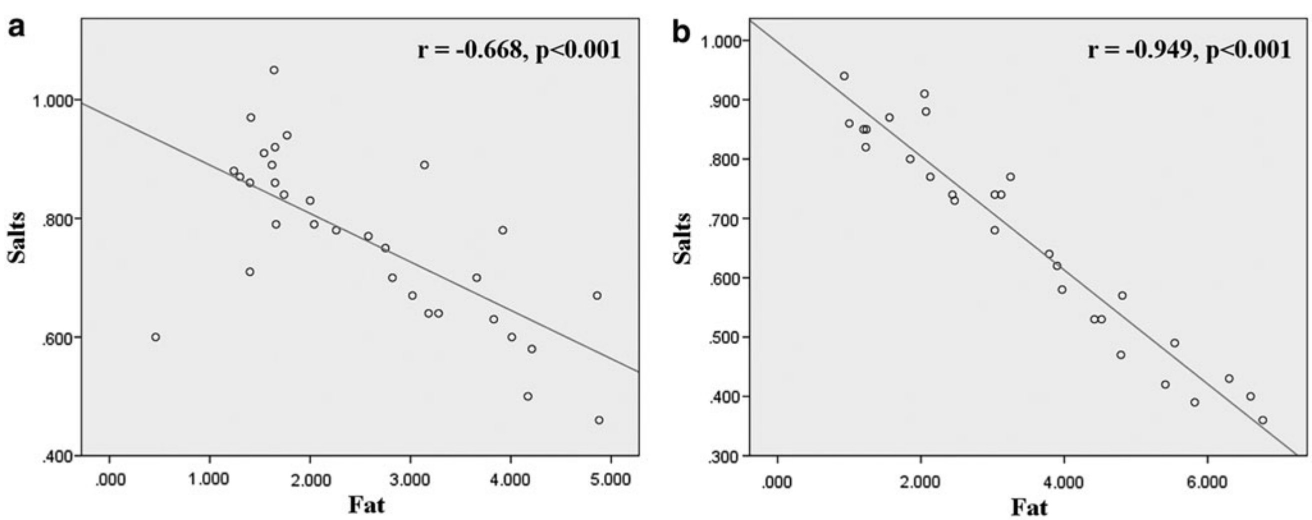

FIG. 2. The correlation between fat and salts in human milk of studied groups. **(a)** There was significant negative correlation between the evaluated factors, salts and fat, in human milk of sons group ($r = -0.668$, $p < 0.001$). **(b)** There was significant negative correlation between the evaluated indices, salts, and fat, in human milk of daughters group ($r = -0.949$, $p < 0.001$). The correlations were analyzed by Pearson's correlation coefficient method.

inducing ductal and alveolar growth of the mammary gland, may also be influenced by fetal sex.[7] These fetal-origin factors may move into maternal circulation and join directly to mammary gland receptors and eventually affect the milk production.[7] After suckling, these hormones and factors in mother's milk may play important roles in the physiology, metabolism, growth, and development of infants.[3] Further precise studies are necessary to fully understand the association of nutritional and hormonal changes with infant sex and subsequent sex-specific growth. The participant lactating mothers in this study may generally have had a more positive attitude toward breastfeeding; therefore, a selection bias might be present. Most of the participants were from East Azerbaijan province of Iran, and it may limit the generalizability of the study results to other, more culturally diverse, populations. Furthermore, small sample size, only one HM sample per mother, no dietary data of mothers, and limited evaluated factors can also be considered as weaknesses of this study. Therefore, further studies with larger sample size are required to evaluate other hormones and factors in HM and correlate them with sex-specific infant growth and development. Because of the impact of maternal diet on the HM components, it is better that the dietary data also be considered in the future studies.

Conclusion

In conclusion, male and female infants may have different nutritional demands for proper growth and development. The results of this study showed that Iranian mothers produced different milk composition for their male and female infants in the case of fat and salt contents. This can be considered as a mechanism for promoting health, growth, and development of infants, and protecting them against probable complications. Knowing these differences can be helpful to devise a sex-specific nutritional strategy to help infants grow up better. However, the results should be confirmed by additional cohort studies.

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Address correspondence to:
Sina Raeisi, PhD
Pediatric Health Research Center
Tabriz University of Medical Sciences
Sheshgelan Street
Tabriz 5136735886
Iran

E-mail: sina_raeisi7007@yahoo.com;
raeisis@tbzmed.ac.ir