RESEARCH BRIEF

Changes in Preterm Human Milk Composition with Particular Reference to Introduction of Mixed Feeding

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Objective: To study the changes in composition of preterm milk till 6 months of age. **Methods**: Milk samples from 33, 19, 7 and 12 lactating mothers (delivered <34 weeks) were analyzed on days 7, 28, 90 and 180, respectively. **Results**: Triglyceride and sodium concentrations increased significantly with time and protein concentration decreased significantly over 180 days. Sodium (*P*=0.02) and triglyceride concentrations (*P*=0.06) were higher in milk samples of mothers who had introduced mixed feeding by 6 months post natal age (*n*=6) compared to exclusively breast-feeding mothers (*n*=6); but lactose and protein content was not significantly different. **Conclusions**: Milk of preterm mothers has higher amount of triglycerides and sodium during introduction of mixed feeding.

Preterm human milk has higher content of protein, electrolytes, vitamins, minerals and anti-infective factors compared to term milk [1-4]. However, there is paucity of data on composition of preterm human milk beyond the first few months until introduction of complementary feeding.

Earlier studies showed that the milk composition of term mothers changes significantly during introduction of complementary feeding [5-8]. The objective of this pilot study was to describe the change in concentration of protein, triglycerides, lactose, sodium, potassium, calcium and phosphorus in preterm milk until introduction of other feeds at 6 months.

METHODS

This longitudinal study was conducted in a neonatal intensive care unit in Northern India after obtaining approval from Institutional ethics committee.

Mothers of inborn singleton preterm neonates (gestation <34 weeks) were enrolled – after obtaining informed consent – if they were feeding breast milk (directly or expressed). Mothers who started other milk or feeds before 4 months of postnatal age were excluded. Mothers were counseled by a nurse certified in lactation counseling. The clinical profile of the mothers and infants was recorded.

Breast milk samples were collected at 7, 28, 90 and 180 days of life, with a margin of ± 2 days. All samples were collected between 2-4 PM; 5 mL milk was manually expressed into wide-mouthed, acid-washed, freezer-safe, plastic vials after the infant had nursed for 3 minutes, or after expressing for 3 minutes, whichever was applicable [5]. The plastic vials were stored at -20°C until analysis. Maximum duration of storage until analysis was 4 months. Proteins were analyzed by micro-Kjeldahl method, triglycerides by Roese-Gottlib method, lactose by enzymatic hydrolysis; sodium, potassium and calcium by ion selective electrodes and phosphorus by ammonium molybdate method [9-11]. Triglycerides were assayed as a surrogate for milk fat content as 98% of breast milk fat is triglycerides [12].

A sample size of convenience was enrolled over one year. Repeated measurements were analyzed by mixed linear models. Normally distributed numerical variables were compared by Students t-test and skewed variables by Mann-Whitney U test.

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RESULTS

Milk samples were collected from 33, 19, 7 and 12 mothers on days 7, 28, 90 and 180, respectively. The decline in numbers were due to inability to report on the sampling days, withdrawal of consent mid-way through the study, introduction of supplementary milk, and loss to follow-up. Exclusive breastfeeding rates were 97%, 100%, 100% and 50%, respectively. Sometime between 4 months and 6 months (day 180), six mothers had introduced mixed feeds and supplementary milk. The 33 infants had mean (SD) birthweight of 1294 (262) g and a mean (SD) gestational age of 31.4 (2.3) wks. The mothers of these infants had a

TABLE I CHANGE IN COMPOSITION OF PRETERM HUMAN MILK OVER FIRST 6 MONTHS OF LIFE

Nutrient	Day 7 (*N*=33)	Day 28 (*N*=19)	Day 90 (*N*=7)	Day 180 (*N*=12)	*P* value
Lactose (g/dL)	6.18 (2.3)	6.66 (1.8)	7.01 (1.3)	5.88 (1.7)	0.289
Triglycerides (g/dL)	3.38 (2.0)	3.82 (1.5)	4.31 (1.1)	5.81 (2.3)	0.001
Proteins (g/dL)	2.13 (0.6)	2.04 (0.7)	1.30 (0.2)	1.35 (0.3)	<0.001
Sodium (mEq/L)	17.4 (11.8)	9.56 (5.3)	13.25 (5)	19.1 (20.9)	<0.001
Potassium (mEq/L)	16.02 (3.9)	14.71 (3.6)	12.6 (3.1)	13.9 (4.3)	0.099
Calcium (mg/dL)	22.3 (12.1)	24.8 (11.8)	20.9 (6.3)	21.3 (10.6)	0.181
Phosphorus (mg/dL)	10.1 (7.3)	11.8 (6.9)	12.5 (8.6)	9.4 (2.6)	0.180

Values in mean (SD).

mean (SD) height of 153.1 (6.1) cm, mean (SD) postpartum weight on day 7 of 58.2 (12.1) kg, and mean (SD) postpartum body mass index (BMI) of 24.8 (5.4) kg/m^2. Common pregnancy-related morbidity included pregnancy induced hypertension (10), antepartum hemorrhage (1), oligohydroamnios (2), prolonged rupture of membranes (1), and preterm premature rupture of membranes (2).

The lactose concentration increased with postnatal age until day 90; and then declined by day 180 (***Table* I**). On repeated measures analysis, the triglyceride concentration showed a significant increase with postnatal age until day 180 (*P*= 0.001). The protein concentration showed a statistically significant decrease with postnatal age (*P*<0.001). On day 180, sodium concentration was significantly higher in the milk of mothers who had introduced mixed feeding (*P*=0.02) (***Table* II**).

DISCUSSION

We demonstrated a progressive increase in triglycerides and sodium, a progressive decline in protein, and non-significant changes in other nutrients in preterm human milk from 7 to 180 days postnatal age. The milk of preterm mothers, who had introduced mixed feeding after 4 months, had a higher content of triglycerides and sodium.

Overall, these changes are similar to those reported for term mothers who had introduced complementary feeding. Neville, *et al*. [8] reported a significant increase in sodium and protein, and decrease in lactose concentration when the milk output of term mothers fell below 400 mL/day. Garza, *et al*. [7] reported higher protein and sodium levels after introduction of complementary feeding in term-born infants.

The change in composition of preterm human milk during introduction of mixed or complementary feeding may be a result of breast involution and increased inter-cellular permeability [5,8,13,14]. Lack of exclusive breast milk feeding causes milk stasis – an important stimulus for opening of tight junctions and increasing permeability. The change in composition may provide a survival advantage to the infant.

The limitations of the study included a small sample size, loss of patients during follow-up, inability to draw a milk sample representative of the entire breast-feed, and lack of anthropometric data on the infants at 6 months and its correlation with breast milk composition. Storage of milk in the freezer compartment up to a maximum of 4 months may have altered the composition of the milk samples to variable extents.

We conclude that the composition of preterm human milk changes around the period of introduction of mixed feeding at about 6 months. If these preliminary findings are confirmed in larger studies it would imply that akin to colostrum – breast milk composition changes according to the needs of an infant who is started on mixed feeding.

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TABLE II MILK COMPOSITION IN MOTHERS EXCLUSIVELY BREASTFEEDING AND THOSE GIVING MIXED FEEDING BY 6 MONTHS POSTNATAL AGE

Macronutrient	*Exclusive breast-fed (n=6)*	*Infants receiving feeds (n=6)*
Lactose (mg/dL)	6.51 (1.6)	5.25 (1.7)
#Triglycerides (mg/dL)	4.28 (1.7)	7.35 (1.7)
#Proteins (mg/dL)	1.28 (0.2)	1.41 (0.4)
*Sodium (mEq/L)	9.87 (9.2)	37.50 (30.4)
Potassium (mEq/L)	14.70 (4.8)	13.25 (3.9)
Calcium (mg/dL)	19.16 (11.1)	23.17 (10.8)
Phosphorus (mg/dL)	10.16 (3.3)	8.80 (2.0)

*Values in Mean (SD); *P=0.02; #P=0.06.*

WHAT THIS STUDY ADDS?

- Milk of preterm mothers who introduce mixed feeding by 6 months has a significantly higher amount of sodium/ compared to that of preterm mothers who continue to exclusively breastfeed.

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