

**Sialic Acid and Iron Contents in Breast Milk of Chinese Lactating Women**

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**ABSTRACT**

**Objective:** To study sialic acid and iron content in breast milk in Chinese women during different lactation stages.

**Methods:** Sialic acid and iron content of colostrum, transitional milk, mature milk, and involutinal milk were determined using a neuraminidase assay kit and the ferrozine method, respectively in 88 lactating women (58 Term, 30 Preterm).

**Results:** The mean (SD) sialic acid levels of colostrum, transitional milk, mature milk, and involutinal milk were 2201.4 (676.6) mg/L, 1445.9 (423.4) mg/L, 395.3 (96.0) mg/L and 273.0 (76.9) mg/L, respectively. The median iron content were 0.05 mg/L, 0.06 mg/L, 0.25 mg/L and 0.35 mg/L, respectively, in successive stages of lactation. Sialic acid and iron were significantly higher in breast milk of preterm mothers compared to term mothers.

**Conclusion:** Sialic acid and iron content in breast milk vary greatly throughout the lactation stages, which probably reflects the infants' needs for growth and development at different stages.

**Keywords:** *Colostrums, Composition, Human milk, Lactation.*

Sialic acid (SiA) is essential for the synthesis of gangliosides which are major constituents located on the surfaces of cerebral cortex cells and play an important supporting role in the differentiation and proper functioning of nerve cells [1,2]. Adequate supplementation of SiA in food rapidly increases SiA content in the cerebral cortex early in life [3], which further improves learning and memory of the developing brain [3,4]. Breast milk is the main exogenous source of SiA for infants. It mainly presents in bound form inside the oligosaccharides, glycoproteins, and glycolipids [5]. Breast milk is also the infant food from which iron is most efficiently absorbed, and levels of serum iron, serum ferritin and red cell folate are significantly higher in breastfeeding infants as compared with those fed cow's milk [6]. There is insufficient evidence to support supplementation on iron in healthy normal weight infants [7]. This study investigated SiA and iron content in breast milk from Chinese post-partum women during different stages of lactation.

## METHODS

This was a prospective observational study conducted at the affiliated Hospital of Hangzhou Normal University from January 1, 2015 to August 31, 2015. Healthy post-partum mothers who consented to donate breast milk for analysis were enrolled into the study. Initially 5 mL colostrum was collected, and thereafter the mothers were followed and 5mL milk was collected at each the following post-partum days – 7-9 days (transitional milk ), 90-100 days (mature milk) and 300-365 days (involutional milk). All samples were collected by pressure suction and the first 5 mL milk during breastfeeding was used for analysis. This was transferred into a sterile tube, followed by centrifugation (10,000 rpm, 30 min) to separate milk into three layers: upper layer of milk fat, middle layer of transparent whey, and white precipitates of casein. The whey layer was then collected and stored in  $-70^{\circ}\text{C}$  for later analysis. Only breast milk samples from women who had provided colostrum, transitional milk and mature milk were used for the analysis. Written informed consent was obtained from all participants. The study was approved by the ethics committees of Children's Hospital, Zhejiang University School of Medicine and the Affiliated Hospital of Hangzhou Normal University.

SiA content in breast milk was measured using a neuraminidase assay kit (Beijing Jiuqiang Biotechnologies, Inc., Beijing, China) and the iron content was measured using the ferrozine method (Ningbo Medical Biotechnology Co., Ltd., Ningbo, China). Both assays were read by Olympus AU 5400 automated biochemical analyzer (Olympus, Japan).

*Statistical analysis:* SiA concentration, gestational age and birth weight are presented as mean (SD); iron contents were presented as median. One-way ANOVA was used for comparison of SiA and iron content between groups. Pearson's correlation analysis was used to study the relationship between SiA or iron content and time of lactation.  $P < 0.05$  was considered as statistically significant difference.

## RESULTS

Eighty-eight women provided donated colostrum, transitional milk, and mature milk during the study period, while 60 women also provided involutional milk. All mothers had given birth to singletons (45 males, 51.1%). Thirty-two (36.4%) had delivered by vaginal route. The gestational age of the infants ranged from 199–291 days with a mean (SD) of 263.6 (20.3) days. There were 30 preterms births at a

mean (SD) gestation of 242.1 (19.1) days. The birth weight of the infants ranged from 1.29–4.72 kg and the mean (SD) was 3.05 (0.75) kg (19 infants were low birth weight).

**Fig. 1** shows the content distribution of SiA (mg/L) and iron (mg/L) in breast milk. The mean (SD) SiA content of colostrum, transitional milk, mature milk, and involucional milk were 2201.4 (676.6) mg/L, 1445.9 (423.4) mg/L, 395.3 (96.0) mg/L, and 273.0 (76.9) mg/L, respectively. SiA content was negatively correlated with the postpartum days ( $R=-0.677$ ,  $P<0.001$ ). The median iron content were 0.05 mg/L, 0.06 mg/L, 0.25 mg/L, and 0.35 mg/L, respectively, in colostrum, transitional, mature and transitional milk. On the other hand, iron content was positively correlated with the postpartum days ( $R=0.773$ ,  $P<0.001$ ). **Table I** shows the comparison of SiA and iron in the breast milk between mothers of the full-term and the preterm infants.

## DISCUSSION

In the present study, SiA content in colostrum was the highest, and it declined successively throughout the lactation and was negatively associated with postpartum days. SiA content of milk (except colostrum) was significantly higher in the preterm mothers than in the full-term mothers. Iron content in colostrum and transitional milk was very low and increased successively throughout the lactation. It had a positive correlation with postpartum days. Iron contents of mature milk and involucional milk were significantly higher in preterm mothers than in the full-term mothers.

In most women, colostrum production on days 1–3 was small. Potential variations of composition may exist because of the small volume of milk obtained [8], which was a limitation of the study.

SiA is highly abundant in colostrum. SiA content in breast milk rapidly declines with prolonged lactation, which reaches 80% reduction by the third month postpartum [9]. Wang *et al* found that the SiA content in the breast milk of preterm mothers was higher than that in full-term mothers, [9] which was consistent with the finding in our study. The iron content in breast milk varies greatly in different populations and geographical regions [10,11]. A study in Korea [10] measured the trace elements in the breast milk of 96 women on postpartum days 5–15 and showed that iron concentration was 5.85 (8.53) mg/L. However, Maru *et al.* [12] found that iron content of colostrum from women in Ethiopia on postpartum day 4 was 0.50 (0.08) mg/L, which is consistent with our findings.

Breastfeeding as early as possible provides rich SiA and facilitates the rapidest brain development in newborns [13]. Whereas extended breastfeeding helps to replenish iron content and prevent iron deficiency anemia when reserve iron was gradually consumed and blood volume rapidly increased, which is especially important for preterm infants to facilitate their catch-up growth. Therefore, breast milk is the most ideal food for infant and breastfeeding should be maintained for a prolonged period.

#### WHAT THIS STUDY ADDS?

- Sialic Acid content in colostrum is high, and it declines with increasing post-partum days of lactation.
- Iron content in colostrum and transitional milk were very low, and increase successively throughout the lactation.
- Sialic acid and iron content is higher in breast milk of preterm mothers than full-term mothers.

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*Contributors:* WHJ was responsible for obtaining consent, following up the subjects collection of milk, & estimation of sialic acid levels, and drafting the article. HCZ was responsible for the design of the study; acquisition of clinical data; analysis and drafting the article, revising the article. RLL was responsible for obtaining consent, collecting colostrum and transitional milk, and revising the article. HLQ estimates the concentration of iron and revised the article. SSQ was responsible for obtaining consent, and collecting colostrum and revising the article.

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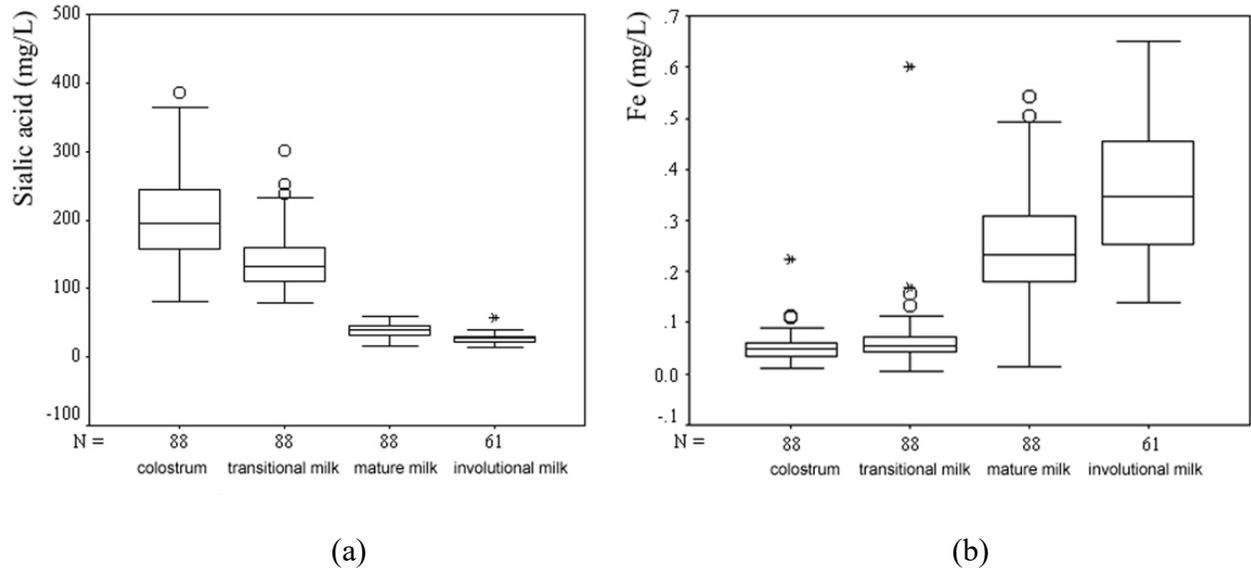
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**TABLE I** COMPARISON OF SIALIC ACID AND IRON CONTENT (mg/L) OF MILK BETWEEN FULL-TERM MOTHERS DELIVERING AND PRETERM INFANTS

	<i>SiA concentration (mean, SD) (mg/L)</i>		<i>Iron concentration (mean, SD) (mg/L)</i>	
	<i>Full-term Mothers</i>	<i>Preterm Mothers</i>	<i>Full-term Mothers</i>	<i>Preterm Mothers</i>
	<i>(n=58)</i>	<i>(n=30)</i>	<i>(n=58)</i>	<i>(n=30)</i>
Colostrum	2161.0 (695.5)	2302.6 (609.0)	0.05 (0.04)	0.05 (0.03)
Transitional milk	1361.7 (413.7)	1556.3 (420.4) <sup>b</sup>	0.06 (0.06)	0.06 (0.03)
Mature milk	376.6 (92.4)	424.7 (95.1) <sup>b</sup>	0.23 (0.10)	0.27 (0.08) <sup>b</sup>
Involutional milk	262.1 (72.1)	317.4 (82.9) <sup>b</sup>	0.34 (0.13)	0.44 (0.14) <sup>b</sup>

<sup>a</sup>Only, forty-nine full-term mothers and 12 preterm mothers provided involutional milk samples <sup>b</sup> $P < 0.05$



**FIG 1.** Box plots of SiA (a) and iron (b) content in colostrum, transitional milk, mature milk, and involutinal milk.