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ORIGINAL ARTICLE

Changes in Preterm Breast Milk Nutrient Content in the First Month

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Key Words
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Background: The primary aim of the study was to investigate the changes in composition of breast milk from mothers with preterm infants (gestation age < 35 weeks) during the first 4–6 weeks of lactation.
Methods: Breast milk from 17 mothers who had delivered preterm infants was collected longitudinally for 4–6 weeks. Breast milk from 15 mothers of full-term infants was also collected at the 1st week and 4th week. Fat, protein, lactose, energy, minerals (calcium and phosphate), and immune components [secretory immunoglobulin A (IgA), leptin, lysozyme, and lactoferrin] content were measured weekly in each participant. A mid-infrared human milk analyzer was used to measure the protein, fat, and lactose contents. Calcium and phosphate components were checked via spectrophotometry. The concentrations of major immune components (secretory IgA, lactoferrin, lysozyme, and leptin) were quantified using enzyme-linked immunosorbent assay kits.
Results: Eighty samples from 17 preterm mothers were collected. The mean gestational age was 29.88 ± 2.39 weeks. There were significant changes in nutrient components during these periods, with increases in lactose ($p < 0.001$), lipid ($p = 0.001$), calorie ($p = 0.012$), and phosphate ($p = 0.022$) concentration and decreases in protein ($p < 0.001$) and secretory IgA ($p < 0.001$) concentration. There were no differences in calcium ($p = 0.919$), lactoferrin ($p = 0.841$), leptin ($p = 0.092$), and lysozyme ($p = 0.561$) levels. Furthermore, there were no significant differences in most components of breast milk between full-term and preterm mothers.
Conclusion: The longitudinal study revealed significant changes in macronutrient contents and secretory IgA concentration in preterm milk over the 4–6 week period, which is compatible with

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the results of previous studies. The quantification of phosphate in preterm breast milk was lower than the normal range, suggesting that close monitoring of body bone mass may be indicated. More studies are warranted to evaluate the clinical significance of alterations of major milk components during the postnatal stage.

1. Introduction

Human milk is the standard for infant feeding and nutrition according to the statement of the American Academy of Pediatrics.[1] It contains various nutrients, enzymes, hormones, host defense agents, and so on. These components are influenced by many factors, including gestational age,[2] lactation stage,[2,3] mother's body mass index (BMI),[4] parity number,[5] diurnal variations,[6] and even the material of containers.[7]

It is important to provide sufficient nutrients to support the extrauterine rate of growth and adequate neurodevelopment of preterm infants.[8] Recent studies on the effect of early postnatal nutrition reveal that the rate of body weight gain of preterm infants is affected by the quantity of calories in the diet; the gains in head circumferences and body length are influenced by the amount of protein given.[9] Other studies have proved that human milk provided to preterm infants has numerous beneficial effects, such as improvements in host defense, gastrointestinal function, and neurodevelopment.[8] In clinical practice, most clinicians suggest fortification of human milk with artificial supplement, such as the human milk fortifier, to fulfill the increased nutritional needs of preterm infants.[10] However, because of the gastrointestinal immaturity of premature infants, it is important to consider whether the composition of these supplementary nutrients, as well as the osmolality, are appropriate.[11] Therefore, it is important to precisely quantify the nutrient components given to preterm infants.

Longitudinal analyses of these nutrient components are scarce and diverse. Thus, the primary aim of the present study was to investigate the changes in the composition of breast milk from mothers with preterm infants whose gestational age was $<$35 weeks during the first 4–6 weeks of lactation. We analyzed the levels of macronutrients, calcium and phosphate, and the bioactive proteins, including leptin, secretory IgA, lactoferrin, and lysozyme.

2. Materials and methods

The study was conducted at Taichung Veterans General Hospital, Taichung, Taiwan, between September 2012 and January 2013. It was approved by the Institutional Review Board of Taichung Veterans General Hospital. Informed consent was obtained from all participants before the study.

2.1. Milk samples

A total of 80 fresh human milk samples were collected from 17 healthy lactating mothers who gave birth to preterm infants, gestational age $<$ 35 weeks and birth weight $<$ 2000 g. Each participant gave 20 mL breast milk each time on Days 5–7, Days 12–14, Days 19–21, Days 26–28, Days 33–35, and Days 39–42 after delivery, as long as she was lactating. Milk was obtained by hand or pump expression. After each expression, the samples were immediately poured into the container provided and stored in the refrigerator in order to prevent the difference between foremilk and hindmilk. Each participant was taught the collecting techniques by our nurses who were all qualified as lactation consultants. Thirty fresh human milk samples were also collected from 15 healthy lactating full-term mothers on Days 3–7 and Days 28–35 after delivery and served as the control group. All samples were processed and analyzed within 24 hours. In order to keep a good relationship with the volunteer mothers, we kept contacting them by telephone at the collect period and the first author would visit the mother after they were discharged.

2.2. Procedures

Each human milk sample was divided into three parts. Calcium and phosphate components were analyzed by spectrophotometry of 5 mL milk. Another 5 mL milk sample was used to measure the components of fat, protein, lactose, and energy by the mid-infrared human milk analyzer, as described in our previous study.[7] Before using the analyzer, the breast milk was heated to 40°C in a water bath and homogenized in the container. Each sample was analyzed twice, and the average value was used for further analysis. A sample of the breast milk (10 mL) was used to check the concentrations of secretory immunoglobulin A (IgA), lactoferrin, lysozyme, and leptin using the same methods described previously.[12]

2.3. Statistical analysis

The Kruskal-Wallis test was used to test the differences between groups. Infants' gestational age and birth weight were included in the analysis and changes in nutrient components were determined by a multiple regression test. All statistical analyses were performed using SPSS version 13 (SPSS Inc., Chicago, IL, USA). Significant differences were defined as $p < 0.05$.

3. Results

3.1. Demographic characteristics of mothers

A total of 110 milk specimens were collected in the study, including 80 samples from preterm mothers and 30 samples