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Breast milk retinol concentration in mothers of preterm newborns

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ABSTRACT

Background: Preterm newborns have low vitamin A reserves at birth, which increases their risk of morbidity and mortality. In the absence of supplementation, breast milk is the only source of this nutrient for exclusively breastfed infants.
Aims: To assess retinol concentration in preterm milk and the relationship between this retinol concentration and lactation phase, degree of prematurity, and maternal serum retinol level.
Study design: Longitudinal study.
Subjects: Fifty-eight preterm mothers.
Outcome measures: Colostrum (1–3 d), transitional (7–15 d), and mature human milk (30–55 d) samples were collected. Maternal blood was collected once at postpartum. Retinol level was measured using high-performance liquid chromatography.
Results: Milk retinol concentration was statistically different between lactation phases ($p < 0.001$): 2.84 ± 1.05 μmol/L in colostrum (58), 3.47 ± 1.28 μmol/L in transitional (58), and 2.03 ± 0.61 μmol/L in mature milk (30). No difference was found in milk retinol levels between groups with different degrees of prematurity ($p > 0.05$). Maternal serum retinol (1.82 ± 0.50 μmol/L) did not correlate with milk levels ($p > 0.05$).
Conclusions: Retinol level in preterm milk seems to be independent of the degree of prematurity and maternal serum status. A significant increase in micronutrient levels in transitional milk was observed, which is likely to contribute to reserves in the premature liver.

1. Introduction

Premature birth is characterized by birth before the 37th gestational week. It is the leading cause of death among neonates and children aged 1–59 months [1,2]. Preterm birth also increases the risk of neonatal death due to other causes, especially infections. Many infants that survive require special care and are at higher risk of health problems, such as cerebral palsy, intellectual deficiencies, chronic pulmonary disease, and visual and hearing losses [3].

The special care required by preterm newborns includes nutritional support and the best choice for this is breastfeeding. Breast milk is beneficial to the health of these neonates and reduces infection-related morbidity and mortality [4].

Besides the number of macronutrients in breast milk, which are important for growth and weight gain in preterm newborns, some micronutrients, such as vitamin A, play essential roles in the health of these newborns. Beneficial effects of vitamin A are due to its ability to promote growth and differentiation of epithelial tissues including those of the skin, intestines, and lungs. Vitamin A also plays a role in the immune system as well as eyesight function [5]. Therefore, if the developmental nutritional needs of this micronutrient are not met, there is an increased risk of deficiency, which predisposes the child to bronchopulmonary dysplasia, respiratory difficulties, retinopathy of prematurity, and infectious diseases that are commonly observed in preterm newborns [6].

Breastfed newborns and breastfeeding mothers are considered risk groups for vitamin A deficiency [7]. This situation is more critical in preterm neonates because of the early interruption of the placental retinol transference to the fetus [8]. These babies show lower plasma levels of retinol and retinol binding protein (RBP) at birth when compared with full-term newborns [6].

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In order to build liver reserves and ensure a proper retinol nutritional status, breast milk must contain adequate amounts of vitamin A for the infant, especially in the first few months after birth. However, longitudinal studies on breast milk retinol content in mothers of preterm infants are scarce.

The purpose of this study was to assess the breast milk retinol concentration in mothers of preterm infants and assess the relationship between this concentration and the phase of lactation (colostrum, transitional milk, and mature milk), the degree of prematurity, and maternal serum retinol levels.

2. Subjects and methods

2.1. Study design and population

This was a longitudinal study carried out with parturients who delivered preterm babies at the Januário Cicco Maternity School, Natal, Brazil.

Sample size was calculated using G*Power 3 software. For a 95% significance level, power of 80%, and expected effect of 0.25 (average effect per convention), the total sample should be composed of 28 cases [9].

Therefore, we recruited 58 volunteer mothers aged between 18 and 40 years. They each gave birth to one single newborn, without malformations, and under 37 weeks of gestation. These data were obtained using the date of the last menstrual period, ultrasounds, or the Capurro method [10]. Women with syphilis or HIV and women who consumed alcohol, tobacco, or supplements containing vitamin A during pregnancy or postpartum were not included.

Socioeconomic status and prenatal and postnatal information were obtained from the prenatal follow-up card, mothers' and babies' medical records, and through interviews with participants.

This study was approved by the Research Ethics Committee from the Federal University of Rio Grande do Norte, CAAE 19864513.7.000.0.5537, statement 461.771. Written informed consent was obtained from all women.

2.2. Sample collection

Within 72 h of giving birth, 2 mL samples of colostrum ($n = 58$) and 5 mL samples of maternal venous blood ($n = 58$) were collected. Between the 7th and 15th days after delivery (transitional phase of lactation), mothers underwent a new collection of 2 mL of breast milk ($n = 58$).

Between the 30th and 55th days after delivery, 2 mL of mature milk were collected in the hospital ($n = 8$), at the mothers' home ($n = 9$), or during ambulatory follow-up consultations ($n = 13$). During this last period, 48% of the subjects ($n = 28$) were lost to follow-up because of the following reasons: mothers were discharged and lived far away, or in a different city ($n = 16$); mothers were discharged and could not be contacted ($n = 8$); milk secretion stopped ($n = 2$); mothers were transferred to a different hospital ($n = 2$).

Milk was collected manually during the fasting period, from a breast not previously used for feeding on the collection day; the first jets were discarded to avoid variations in fat and retinol amounts. The samples were stored in identified tubes protected from light exposure. Biological materials were transported under refrigeration to the laboratory, where blood was centrifuged ($500 \times g$) for 10 min and 1 mL of serum was separated for analysis. Aliquots of 500 μL of colostrum and 1 mL of transitional and mature milk were also separated, and all samples were stored at −20 °C until analysis.

At the end of the study, participants received a megadose of 200,000 IU (60 mg) of vitamin A as recommended by the Brazilian Ministry of Health [11].

2.3. Biochemical analysis

The extraction methods of retinol from serum and milk were adapted from the study by Ortega et al. [12] and Giuliano et al. [13], respectively. To precipitate the proteins, an equal volume of 95% ethanol was added to each biological sample. In addition, an equal volume of 50% potassium hydroxide was added to milk for alkaline hydrolysis, performed in a 60 °C water bath for 60 min. Hexane was used as an extractive reagent. Three washes were carried out with 2 mL of hexane. One minute of agitation and 10 min of centrifugation ($500 \times g$) were performed to separate and remove the hexane layer. The total hexane extract (6 mL) was agitated, and 3 mL were evaporated in a 37 °C water bath. The dry extract was diluted in 250 μL of absolute ethanol and 20 μL were analyzed by high-performance liquid chromatography (HPLC).

Levels of retinol were determined in a LC-20 AT chromatograph, with a loop injector of 20 μL, CBM 20A communicator, and SPD-20A UV-VIS Detector with UV detection ($\lambda = 325$ nm) (Shimadzu Corporation®). Chromatographic separation was performed in a Luna 5u C18(2) 100A phenomenex® Reversed Phase Column, 250 mm × 4.6 mm. Elution was isocratic, with the mobile phase of 100% methanol at 1 mL/min flow. LC Solution® software was used to visualize the chromatograms.

A standard solution of retinol (Sigma®) [with the concentration confirmed by the specific coefficient of extinction in absolute ethanol (ε 1%, 1 cm = 1780)] was previously applied to all analyses [14]. The identification and quantification of vitamin A in the samples was performed by comparing the retention time and the peak area to those of the standard.

2.4. Reference values

Classification of the degree of prematurity was made according to gestational age: extremely preterm (<28 weeks), very preterm (28 to 32 weeks) and moderate to late preterm (32 to 37 weeks) [3].

The cutoff to identify serum vitamin A deficiency in breastfeeding mothers was 0.7 μmol/L (20 μg/dL). Levels higher than 1.05 μmol/L (30 μg/dL) were considered adequate in mature milk [7].

2.5. Statistical analysis

The data were analyzed using the statistical software SPSS (22.0 for Windows, SPSS Inc., Chicago, USA). The retinol concentrations in the samples are given as mean ± standard deviation (95% confidence interval). The tests were selected after variables had shown near-normal distribution.

Repeated-measures analysis of variance was used to verify differences between retinol concentration, comparing the phases of lactation and between vitamin A concentrations in accordance with the level of prematurity. The Pearson's r correlation test was used to evaluate the association between gestational age and milk retinol levels, as well as between serum and milk retinol levels. Results were considered significant when $p < 0.05$.

3. Results

The mean gestational age of newborns was 32.1 ± 3.5 weeks and the mean birth weight was 1903 ± 679 g. All babies required intensive neonatal care immediately after birth. Table 1 shows the characteristics of the population under study.

In colostrum, the average retinol concentration was 2.84 ± 1.05 μmol/L (81.4 ± 30.1 μg/dL) ($n = 58$), which increased to 3.47 ± 1.28 μmol/L (99.4 ± 36.7 μg/dL) ($n = 58$) in transitional milk and decreased to 2.03 ± 0.61 μmol/L (58.1 ± 17.5 μg/dL) ($n = 30$) in mature milk. Significant differences were observed in all phases ($F_{(2,58)} = 25.59$, $p < 0.001$) (Fig. 1). Only 1 woman showed retinol levels lower than 1.05 μmol/L in mature milk.

After comparing amounts of vitamin A in milk of extremely preterm ($n = 6$), very preterm ($n = 16$), and moderate to late preterm ($n = 36$) groups, there were no significant differences in breast milk at any