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Research Article

Maternal Vitamin E Nutritional Status and Alpha-Tocopherol Levels in Mature Milk

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Abstract

This study aimed to evaluate the relationship between maternal vitamin E biochemical nutritional status and alpha-tocopherol levels in mature milk. At one-month postpartum, mature milk was collected and analyzed by high-performance liquid chromatography. Analyzing the mothers individually, it was found that 30% of them had serum vitamin E levels below the cutoff point. There was no significant association in alpha-tocopherol concentrations between serum and mature milk. The maternal organism seeks to protect the infant from insufficient vitamin E levels, even when the woman does not present adequate serum levels of this vitamin.

Keywords: *Maternal Vitamin E; Alpha-Tocopherol; Mature Milk*

Introduction

Vitamin E is a general term for eight compounds, in which alpha-tocopherol has the most pronounced biological activity [1] playing an important protective action against damage caused by free radicals [2].

Maternal vitamin E status is directly associated to serum alpha-tocopherol concentrations in the fetus and newborn, thus situations of marginal or low alpha-tocopherol levels in maternal blood may culminate in vitamin E deficiency to mothers and their offspring [3]. However, the effect of maternal status on the vitamin E concentration in human milk during lactation is still under investigation.

Since the vitamin E transport through the placenta is limited, human milk is the only vitamin E source for exclusively breastfed babies; furthermore, human milk is known as the ideal food for infants aged 0-6 months [4]. However, it is known that vitamin E concentrations decrease throughout the lactation period, and that mature milk presents the lowest concentration - highlighting the importance of understanding which factors are associated with this decrease. There are several studies addressing the initial stages of lactation, but there is limited evidence on the latter stages [5].

Considering the importance of evaluating the maternal-child population, the objective of this study was to evaluate the relationship between maternal vitamin E biochemical nutritional status and alpha-tocopherol levels in mature milk.

Materials and Methods

Design and Setting

This longitudinal study was conducted between 2012 and 2013 with 20 volunteer mothers hospitalized in a public maternity hospital located in the city of Santa Cruz, Brazil. Sampling was obtained by spontaneous demand.

This study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte, Brazil, (CAAE 07416912.8.0000.5537). Women recruited for the study were informed about the research objectives and those who voluntarily accepted to participate in the study, signed an informed consent. Socioeconomic characteristics were obtained from the patient records.

Sample

The sample included healthy adult women who did not take supplements containing vitamin E during pregnancy, with singleton, full-term delivery (> 37 weeks of gestation) without complications. Women with clinical conditions (cancer, heart disease, diabetes, gastrointestinal, liver and kidney diseases and infectious diseases), multiple pregnancy and preterm delivery were excluded.

Data collection

Before delivery, fasting blood samples (5 mL) were drawn, and centrifuged for 10 minutes (500 x g) to collect 1 mL of serum from each sample.

Mature milk samples (3 mL) were collected 30 days after delivery under fasting conditions at the hospital or during a home visit previously scheduled with the participant. Milk samples were collected by hand expression of a single breast that had not been previously suckled and the first ejections were discarded to avoid fat-related variations.

Samples were collected in polypropylene tubes wrapped in foil, transported under refrigerated conditions and stored at -20˚C until analyses.

Measurement

Alpha-tocopherol was identified and quantified by comparison of the retention time and the area under the curve to those of the corresponding alpha-tocopherol standard (Sigma®, St Louis, MO, USA). The standard's concentration was confirmed by the extinction coefficient specific to alpha-tocopherol in absolute ethanol (ε 1%, 1 cm = 75.8, at 292 nm) [8].

The socioeconomic data were expressed as absolute and relative frequencies. Alpha-tocopherol levels are shown as mean and standard deviation (SD) in μg/dL. Concentrations of maternal alpha-tocopherol serum levels below 697.7 μg/dL were indicative of low levels of vitamin E [9].

Data analysis

Alpha-tocopherol was extracted from serum and mature milk using an adaptation of the method described by Ortega and collaborators [10]. To the 1000 μL aliquot of serum/milk sample it was added 1000 μL of 95% ethyl alcohol (Vetec®, Rio de Janeiro, Brazil) for protein precipitation. Alpha-tocopherol was then extracted with 2000 μL of hexane (Merck®, Rahway, NJ, USA). The 2000 μL supernatant was then transferred to another tube. This step was done three times, totaling 6mL of supernatant, from which 3 000 μL were extracted after vortex mixing. The excess solvent was evaporated in a 37ºC water bath, being then diluted in 250 μL of ethanol (Analytical grade, Merck, São Paulo, Brazil) and 20 μL was injected to the HPLC equipment - Shimadzu chromatograph with a Shimadzu LC-20 AT pump, Shimadzu SPD-20A/UV-VIS detector (Shimadzu Corporation®, Kyoto, Japan), Phenomenex column (4.6 mm x 15 cm), and LC solution software (Shimadzu Corporation®). The mobile phase used was absolute methanol (J. T. Baker®, Mexico) in an isocratic system with a flow rate of 1.0 mL/minute and wavelength of 292 nm.

Statistical analyses were performed using the Statistica 7 software (StatSoft, Tulsa, OK, USA). The normality of the metric variables of interest was assessed using the Kolmogorov-Smirnov test. The Pearson correlation analysis was used to assess the relationship between alpha-tocopherol concentrations in serum and mature milk, establishing significance at $p < 0.05$.

Results

Most women in the study population were aged in their early twenties, were educated up to high school level and lived with a partner. Most of them were giving birth to their first child and had a low household income. Socioeconomic data are presented in table 1.

Socioeconomic characteristics	n	%
Maternal age		
18 years old	03	15
19 - 23 years old	09	45
24 - 28 years old	06	30
29 - 33 years old	01	05
34 - 38 years old	01	05
Education		
Literate	01	05
Elementary school	04	20
High school (incomplete)	06	30
High school (complete)	06	30
Higher level of education	03	15
Marital Status		
Married or Common-law marriage	12	60
Single	08	40
Children		
One	14	70
Two	04	20
Three	02	10
Family Income		
Up to 1 minimum wage*	11	55
1 to 2 minimum wages	07	35
Socioeconomic characteristics	n	%
2 to 5 minimum wages	02	10

Table 1: *Socioeconomic characteristics of postpartum women attending a public Brazilian maternity hospital (n = 20), 2012/2013.*

Note: Considering the Brazilian minimum wage in 2012 (US$ 330/month).

The overall mean (SD) alpha-tocopherol concentration was 875.0 (350.1) µg/dL, and 30% (n = 6) of them presented low vitamin E levels. Mean (SD) alpha-tocopherol concentration found in mature milk was 399.0 (140.2) µg/dL, and there was no significant association between alpha-tocopherol in maternal serum and the vitamin E concentration in mature milk (R = 0.1747, p = 0.461).

Discussion

Plasma/serum alpha-tocopherol is the most widely used biomarker of vitamin E status, especially because of its practicality and applicability to large surveys [11]. The vitamin E status in the study population was considered normal and the mean concentration (875.0 ± 350.1 µg/dL) was similar to values presented for Brazilian women studied elsewhere (796 ± 312 µg/dL) [12]and Albanian women (862 ± 143.7 µg/dL) [13]; however, it was lower than the values found in other studies conducted in Cuba (1029.4 ± 487.4 µg/dL) [14]and the United States (1438.5 ± 331.6 µg/dL) [15].

In this study, 30% (n = 6) of the lactating women showed low levels of serum vitamin E, yet this result should be cautiously interpreted, given the fact that the sample size was composed by 20 women only. Conversely, a study conducted in Natal, Brazil assessing the maternal population, adopted the same cutoff point for serum alpha-tocopherol (697.7 mg/dL) [16]and it was found a prevalence of 16% of low vitamin E levels among the assessed women, yet that study included 103 women.

Regarding the alpha-tocopherol concentration in mature milk, our findings were similar to those found in Japan and Greece, and higher than the values in Poland [17-19].

In this study, maternal serum alpha-tocopherol levels were not significantly associated with the concentration of this vitamin in mature milk. Most studies evaluating the association between maternal serum vitamin E levels and milk alpha-tocopherol concentration found no association between them [7,20,21]; however, those studies evaluated only colostrum and transitional milk. Despite the few samples, this was a pioneer study in evaluating the association between maternal serum and mature milk, although further investigation is needed.

The non-correlation found in this study corroborates with previous reports indicating that the alpha-tocopherol transfer to the mother's milk does not occur passively, but through controlled mechanisms [22,23] proposed that the mammary gland possibly mobilizes alpha-tocopherol from extrahepatic tissues. In addition to this hypothesis, Traber [24] says that the mammary gland uses the adipose tissue as main source of alpha-tocopherol.

Metabolic and genetic factors that could interfere on the alpha-tocopherol transfer from serum to breast milk and then to the infant's circulation need to be explored by studies including data from pregnancy, lactation, and infancy [25]. Nonetheless, the novel findings of this study initially demonstrate that the mammary gland seems to be able to maintain adequate alpha-tocopherol levels in human milk regardless of maternal serum levels, probably due to the use of mechanisms of transportation independent from maternal serum.

Conclusion

This was the first study analyzing the relationship between maternal serum alpha-tocopherol levels and the amount of this vitamin in the mature milk of mothers attending a public maternity hospital in Brazil. Even though 30% of the participants had low serum alpha-tocopherol levels, there was no significant correlation with the vitamin E concentration in mature milk. The present findings suggest that the maternal organism seeks to protect the infant from low vitamin E levels, even when the woman does not present adequate serum levels of this vitamin.

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Conflict of Interests

The authors declare that there is no conflict of interest.

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