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Vitamin A concentration in mature human milk

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Abstract

Objective: To quantify vitamin A levels in mature milk of 196 nursing women who were treated at the Maternity School of Rio de Janeiro and to evaluate its correlation with sociodemographic variables and degree of nutrition knowledge.

Methods: To quantify retinol concentrations, 10 mL of mature milk were collected by manual expression of one breast, 2 hours after the last feed, in the morning period. Values below 1.05 µmol/L and 2.3 µmol/L were considered inadequate to meet satisfactory intake and to constitute vitamin A liver reserve, respectively. The following variables were also assessed: sex, age, familiar income, maternal education, basic sanitation conditions, number of people in the household, maternal age, prenatal care, and degree of nutrition knowledge.

Results: Among the 196 lactating mothers analyzed, the average vitamin A concentration observed in mature milk was 1.76±0.85 µmol/L and prevalence of vitamin A deficiency was observed in 20.5% mothers. There was no significant difference between vitamin A levels in maternal milk and the variables socioeconomic status and nutrition knowledge. Only 38.9% of lactating women presented enough vitamin A concentrations in milk for the infants' liver reserves (2.3 µmol/L).

Conclusion: These findings reveal high prevalence of inadequate vitamin A nutritional status of mothers and infants, consistent with the national prevalence reported in women of childbearing age and Brazilian children, and that the intervention measures to fight this shortage should be extended to all pregnant and postpartum women, regardless of sociodemographic conditions and degree of nutrition knowledge, in order to improve the health of mother and son.

J Pediatr (Rio J). 2012;88(6):496-502: Vitamin A, human milk, income, maternal education.

Introduction

Vitamin A deficiency (VAD) affects numerous physiological processes such as growth and reproduction, which makes this vitamin an important nutrient during pregnancy and lactation, due to its increased need to meet the demands related to the reproductive process.[1]

Currently, it is known that subclinical VAD increases susceptibility to infections, which can cause immunodeficiency conditions of nutritional origin, besides aggravating cases of diarrhea. It is estimated that every minute a child dies from causes directly or indirectly attributable to VAD.[1]

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At birth, the newborn has low liver reserves of vitamin A because of maternal homeostatic control, which regulates placental transfer of vitamin A to the fetus and prevents high concentrations from being transferred. The newborn's liver storage can be increased during breastfeeding, if the nursing mother presents dietary intake or adequate liver reserve of vitamin A, as well as milk production in adequate concentration and volume.[2]

During the first 6 months of lactation, maternal needs for vitamin A increase, making them larger than those of pregnancy,[3] and their transfer from mother to child increases about 60 times compared to that accumulated by the fetus during pregnancy,[4] becoming the moment of higher nutritional demand of vitamin A.[3]

The content of vitamin A in human milk is variable and possibly influenced by maternal intake and by factors such as age at conception and postpartum, parity and maternal socioeconomic status.[5] Although the literature refers to a possible interference of socioeconomic factors on the concentration of vitamin A in breast milk, there are few studies assessing these associations.

Considering the importance of adequate maternal nutritional status of vitamin A and its impact on infants' health, the present study aimed to describe the vitamin A concentration in mature milk of mothers receiving care at a public hospital in the municipality of Rio de Janeiro, its relation with sociodemographic variables and degree of maternal nutrition knowledge, as a way of contributing to nutritional care and health of mother and child.

Methods

The present work was conducted in a public hospital in Rio de Janeiro, which assists women with similar characteristics to the other pregnant/postpartum adults and adolescents in other health care unities in Rio de Janeiro.

The recruitment of women who had recently given birth occurred in the postpartum follow-up visit. Women who met the inclusion criteria – single pregnancy, delivery at term, not having used supplements containing vitamin A during pregnancy and/or lactation, being in exclusive breastfeeding, and accepting participation by signing the informed consent form – had an appointment scheduled in the Department of Nutrition for referral to the Milk Bank on around the 30th day postpartum and in exclusive breastfeeding. In data collection, performed by a team of trained and supervised researchers, pre-tested instruments were used in a pilot study.

The sample consisted of 196 mothers and their respective infants, assisted in the unit during the period from September 2000 to March 2001, representing 28% of the population treated during the study period.

It was considered exclusive breastfeeding when the infant received only breast milk, directly from the breast or extracted, and no other liquid or solid, except for vitamin drops or syrups, minerals and/or medications.[6]

Mature breast milk samples were collected (10 mL) in the morning, after fasting for at least 8 hours, by manual expression, by the mother, after cleaning the breast with distilled water.[7] The samples were placed in sterile containers and preferably obtained from the breast that was not used in the last feed, keeping an interval of, approximately, 2 hours between the last feed and sample collection.

Milk collection was performed directly in polypropylene bottles with screw cap, wrapped in foil, previously washed and soaked for 24 hours in extra neutral detergent, rinsed with deionized water and placed in nitric acid solution (1:1) for 24 hours.[7] The milk samples collected were subjected to strict control of light and temperature to prevent loss of retinol and carotenoids. Subsequently, milk aliquots were frozen at -20 ºC until the moment of analysis.

To determine retinol concentrations in breast milk, we used the method of high-performance liquid chromatography, according to the technique described by Hess et al.[8] Once retinol is not primary standard, the determination of the concentration of a retinol acetate solution in methanol was conducted, making a standard curve, according to the procedures of quality control recommended by the International Advisory Group on Vitamin A.[9]

For the diagnosis of VAD, the cutoff of < 1.05 µmol/L of milk (30.0 µg/dL) was adopted, suggested by the World Health Organization (WHO).[10] To evaluate the daily intake of vitamin A, the mean volume of daily intake was calculated, based on the average daily caloric needs of infants in the study.

To determine the daily energy requirement, the average weight of infants obtained in the first postpartum follow-up visit was multiplied by 116 kcal, value corresponding to the energy requirement for this age group.[11] Considering that breast milk contains approximately 70 kcal/100 mL[11] and based on the average caloric needs of infants, it was possible to estimate the average daily volume of milk ingested by infants.

From vitamin A concentrations found in breast milk and the average volume of intake by infants, it was possible to estimate the average intake of vitamin A by infants and compare them to the intake proposed by the Institute of Medicine (IOM)[3] and the National Health Surveillance Agency [Agência Nacional de Vigilância Sanitária (ANVISA)],[12] which are of 400 µg retinol (RE)/day and 375 µg RE/day, respectively.

The concentration of vitamin A in breast milk was also evaluated, according to the daily need for liver reserve in newborns. We used the cutoff points suggested by Stoltzfus & Underwood,[4] that indicate a minimum concentration of vitamin A in breast milk of 2.3 µmol/L for liver reserve.

Environmental and sociodemographic variables were obtained through structured questionnaires administered by interviewing the mothers on the following topics: gender, family per capita income, maternal education, number of residents per household, maternal age, and prenatal care.

The level of maternal education was stratified into illiterate, incomplete and complete elementary education, incomplete and complete high school, and higher education.

The monthly per capita income was calculated by adding the individual income of the family members, expressed in two groups: ≤ 1.0 minimum wage (MW) and > 1 MW.

Regarding the conditions of basic sanitation, they were regarded as adequate when the house had water supply with indoor canalization, sewage system, and regular garbage collection, and inadequate when they did not have any of these services.

Pre-natal care was considered adequate if the pregnant woman had participated in six visits or more during gestation.[13]

Nutrition knowledge was assessed through questioning the mothers about foods that should not be missing in their child's diet and about the inclusion and exclusion of foods during pregnancy and lactation.[14] In the analysis of nutrition knowledge, the foods mentioned were compared, considering the food groups suggested by the Daily Food Guide by the United States Department of Agriculture, used as a practical guide for the planning of meals for healthy individuals.[15] These groups were divided into: 1) Milk group: milk and dairy products; 2) Meat group: meat, poultry, fish, sausages, giblets, eggs, legumes and oilseeds; 3) Fruit and Vegetables group: green leafy vegetables, roots, stems or bulbs and fruits; 4) Breads and cereals group: rice, wheat, oats, barley, corn, rye, flour and derivatives.

We also investigated the presence of dietary restrictions and taboos during pregnancy and lactation, through questionnaires with open-ended questions, in which the mothers answered about habits and restrictions during the periods of interest.

The level of knowledge was categorized as: good (mentioning three or more food groups necessary to compose a balanced diet and no food taboos), regular (two or three food groups, with repetition of the same food group and/or mentioning food taboos), and insufficient (only one food group, the same groups of foods and food taboos).[14]

The study was conducted according to the ethical guidelines of the National Health Council[16] after approval of the Research Ethics Committee of Escola Paulista de Medicina from Universidade Federal de São Paulo (protocol n. 435/01). Participant mothers signed an informed consent form.

The present study administered Student *t* test for the assessment of equality of means and chi-square test to verify associations between categorical variables. The level of significance established was of probability lower than 5%. The analyses were performed using SPSS for Windows version 13.

Results

The study included 196 mothers, with mean age of 27.45±6.8 years, where 78.5% were in the age range of 20 to 35 years old. Among the newborns, 54.6% were male and 45.4% female.

The distribution of the sample according to socioeconomic variables is described in Table 1. Most mothers had prenatal care (97.9%), with a mean of 8.98±3.47 visits, but only 6.4% received nutritional counseling.

The mean concentration of vitamin A in mature milk was of 1.76±0.85 µmol/L, with prevalence of 20.5% VAD in mothers and their respective infants, according to the indicator of concentration of vitamin A in maternal milk. There was no significant difference between the concentration of vitamin A in maternal milk and the variables maternal age, maternal education, total and per capita family income, number of people per household, and number of prenatal care visits (Table 2).

Infants had a mean weight of 4,291.31±474.79 g, and upon this weight the mean volume of maternal milk intake was estimated, being of 710 mL/day.

Table 1 - Distribution of mothers who attended the Maternity-School of Universidade Federal do Rio de Janeiro, according to socioeconomic variables

Variables	n	%
Maternal age (years)		
20-35	154	78.6
≥ 35	42	21.4
Education		
Incomplete elementary school*	78	39.8
Incomplete high school†	94	47.9
Higher education‡	24	12.2
Per capita family income		
≤ 1 MW	103	52.5
> 1 MW	93	47.5
Sanitation		
Adequate	138	78.8
Inadequate	37	21.2
People per household		
≤ 4	155	79.1
> 4	41	20.9

MW = minimum wages.
* Illiterate and incomplete elementary school.
† Complete elementary school and incomplete high school.
‡ Complete high school and higher education.

The average daily intake of vitamin ingested by infants was 357.03±172.43 µg. More than half the infants (50.4%) ingested an amount of vitamin A below the daily recommendation proposed by the IOM,[3] and almost half the infants (46.4%) had vitamin A below the recommendations, according to ANVISA.[13] There was no association between the mean intake of vitamin A by infants and maternal sociodemographic variables (Table 3).

Regarding the concentration of vitamin A in the milk needed for liver reserve, there was a prevalence of inadequacy of 61.1%. There was no significant association between sociodemographic variables and vitamin A adequacy in the milk for liver reserve. (Table 4).

Regarding nutrition knowledge, 39.4% were classified as having good knowledge, 30.7%, regular and 29.9%, insufficient. There were no significant differences regarding the concentration of vitamin A in the milk of lactating mothers, according to the level of nutrition knowledge (p = 0.35).

Discussion

VAD is considered a major public health problem in women of childbearing age, manifesting, mainly, during pregnancy and lactation. This deficiency reduces the availability of this nutrient in breast milk, the only food source for infants under exclusive breastfeeding.[17]

The mean concentration of vitamin A observed was of 1.76±0.85 µmol/L, being higher than the average found by Menezes & Trugo[18] and Góes et al.,[19] in studies conducted in mature milk of Brazilian lactating women, whose mean concentration was 1.4±0 µmol/L and 1.51 µmol/L, respectively.

When the concentration of vitamin A in maternal breast milk is less than 1.05 µmol/L, the infant's body reserve may be under the critical concentration estimated (17.5 µmol/g) for physiological requirements needed in the second half of childhood.[2] At birth, the newborn liver reserves of vitamin A are extremely low compared to postnatal requirements of this vitamin, and it is sufficient only for the first days of life, even when maternal reserves are adequate.[20]

In the present study, only 38.9% of lactating women presented enough vitamin A concentration in the milk for liver reserves in newborns (≥ 2.3 µmol/L). Similar results were observed in the study by Mello-Neto et al.,[21] conducted in the state of São Paulo, where only 50% of lactating mothers presented adequate concentrations of vitamin A in the milk. This fact is noteworthy, since some diseases, such as infections, are able to increase the nutritional demand of this vitamin, and if there is not a proper liver reserve, VAD may occur and, consequently, all the negative outcomes of this deficiency.

The average daily intake of vitamin A by infants was of 357.03 µg retinol/day, representing a high prevalence

Table 2 - Vitamin A concentration in mature milk of mothers assisted at the Maternity-School of Universidade Federal do Rio de Janeiro, according to maternal socioeconomic variables

Variables	Mean of retinol (µmol/L)	SD	p
Maternal age (years)			
20-35	1.69	0.75	0.24
> 35	1.89	0.82	
Education			
Incomplete elementary school*	1.84	0.80	0.54
Incomplete high school†	1.73	0.81	
Higher education‡	1.96	0.79	
Per capita familiar income			
≤ 1 MW	1.86	0.88	0.34
> 1 MW	1.75	0.79	
Sanitation			
Adequate	1.80	0.85	0.90
Inadequate	1.81	0.88	
People per household			
≤ 4	1.77	0.68	0.72
> 4	1.69	0.71	

MW = minimum wages; SD = standard deviation.
* Illiterate and Incomplete elementary school.
† Complete elementary school and incomplete high school.
‡ Complete high school and higher education.

of infants with low intake of vitamin A according to the IOM[3] and ANVISA,[12] being this prevalence 5.4 and 46.47%, respectively. This result differs from the findings of Mello et al.,[1] who found adequate intake of this vitamin by infants according to the recommendation of the IOM.[3]

However, the study mentioned was conducted using colostrum, milk that is richer in vitamin A when compared to mature milk. Colostrum is secreted only in the first days of lactation, while mature milk is secreted after the third week postpartum, when vitamin A concentration becomes

Table 3 - Mean daily intake of vitamin A in infants at the Maternity-School of Universidade Federal do Rio de Janeiro, according to maternal socioeconomic variables

Variables	Mean daily intake of vitamin A (mg RE/day)	SD	p
Maternal age (years)			
20-35	342.83	152.14	0.24
> 35	383.40	166.34	
Education			
Incomplete elementary school*	373.25	162.28	0.54
Incomplete high school†	350.94	164.31	
Higher education‡	397.60	160.25	
Per capita family income			
≤ 1 MW	396.50	187.58	0.33
> 1 MW	372.97	169.39	
Sanitation			
Adequate	365.14	172.43	0.90
Inadequate	367.17	178.51	
People per household			
≤ 4	359.05	137.94	0.72
> 4	342.83	144.03	

MW = minimum wages; RE = retinol; SD = standard deviation.
* Illiterate and Incomplete elementary school.
† Complete elementary school and incomplete high school.
‡ Complete high school and higher education.

Table 4 - Adequacy of vitamin A concentration in mature milk of lactating mothers for a liver reserve in infants according to maternal sociodemographic variables

Variables	Adequacy of vitamin A concentration in maternal milk for liver reserve				Total	Chi-square
	Yes		No			
	n	%	n	%		(p)
Maternal age (years)						
20-35	45	29.22	109	70.78	154	2.64
> 35	17	47.22	19	52.78	36	(0.13)
Education						
Incomplete elementary school*	31	38.23	49	61.77	78	
Incomplete high school†	35	33.78	59	66.22	94	1.38
Higher education‡	6	25.00	17	75.00	24	(0.52)
Per capita family income						
≤ 1 MW	42	40.77	71	59.80	103	1.43
> 1 MW	39	41.93	54	58.07	93	(0.52)
Sanitation						
Adequate	46	33.32	92	64.68	138	0.93
Inadequate	9	24.32	28	75.68	37	(0.79)
People per household						
≤ 4	45	29.03	110	70.97	155	2.13
> 4	13	31.70	28	68.30	41	(0.61)

MW = minimum wages.
* Illiterate and Incomplete elementary school.
† Complete elementary school and incomplete higher education.
‡ Complete high school and higher education.

stable and remains this way until the end of lactation. Thus, it is important that the assessment of nutritional status of vitamin A in infants be performed using mature milk.

In the study by Bezerra et al.,[22] the supply of vitamin A through the mature milk of nursing mothers living in the municipality of Natal contributed to supply only 64% of the recommended vitamin A, according to the IOM,[3] even after supplementation.

The literature reports that if the infants' reserves remain low, at the level of liver reserve at birth, the chances of presenting the problems caused by VAD, especially marginal or subclinical deficiency, are greater, particularly in developing countries.[23]

In this study, we observed a 20.5% prevalence of VAD in nursing mothers and their respective babies, which was regarded as moderate by the WHO.[10] Such prevalence becomes even more worrying once the study excluded all preterm infants, as the recognition of lower concentrations of vitamin A in the milk of mothers of preterm newborns is established in the literature, when compared to mothers of term infants.[24] Thus, it is possible that in populations that include both pre-term and term newborns, the prevalence of VAD is even more expressive, making the scenario even worse.

While assessing the epidemiological aspects of VAD, it is observed that, except for the situations of extreme poverty, income and education do not seem to be determining for this deficiency. According to Van Schaik,[25] biologic groups with extremely low socioeconomic level undergo severe nutritional deprivation, but the amount of money spent on food acquisition is not related to the nutritional quality of the diet.

Studies conducted in Rio de Janeiro with pregnant women and preschool children showed that inadequate intake of foods rich in vitamin A is the main etiological factor of deficiency of this vitamin and that its exclusion or low consumption are more related to cultural issues and eating habits than to economic factors.[26]

Santos et al.,[27] while studying the prevalence of VAD among preschool children in the state of Bahia, showed that 69% of the studied population presented inadequate serum retinol levels (< 1.05 µmol/L), and VAD did not show association with maternal education or per capita family income. Similar results were observed by Dimenstein et al.[28] and Vítolo et al.[7] while investigating the association of vitamin A concentrations in the milk of Brazilian nursing mothers with socioeconomic variables.

The lack of association between socioeconomic variables and the concentration of vitamin A in mature milk corroborates the theory that inadequate intake of foods rich in vitamin A would be the main etiological factor of VAD at epidemiological level.[26] Another factor that reinforces this thesis is that the studies that demonstrated the influence of maternal socioeconomic condition on the concentration of vitamin A in milk were conducted primarily in populations in extreme poverty, with high frequency of clinical manifestations of VAD.[28]

It is noteworthy that the low intake of foods rich in this vitamin may not be related to the low nutrition knowledge observed. The lack of association between vitamin A concentrations in breast milk, socioeconomic status, and nutrition knowledge indicates that nursing mothers may be at risk for VAD and reaffirms the need for nutritional interventions in this population group.

Vitamin A supplementation in pregnant women and/or mothers who have VAD gains more space in prenatal and immediate postpartum period, especially when factors that affect dietary intake are present. The results of intervention studies on vitamin A supplementation during pregnancy and postpartum have been encouraging, pointing benefits of meeting the needs of this vitamin.[25,29]

These findings have supported the actions of the Brazilian Ministry of Health, which recommends vitamin A supplements for nursing mothers in the immediate postpartum period and for infants between 6-59 months of age in areas considered at risk for VAD.[30] However, several studies indicate alarming prevalence of VAD outside the coverage area of the program, which need to be considered.[20,26,28]

Another worrying fact is that in Brazil there are no nutritional assistance programs for nursing mothers. During routine visits, attention is directed, in large part, to the infant. It becomes even more important to implement actions on nutritional guidelines, which aim not only to meet the necessary needs of energy-protein of lactation, but also to meet the micronutrients recommendations, especially those with increased requirement during pregnancy and childbirth.

The present findings revealed high prevalence of inadequate vitamin A nutritional status of mothers and infants, consistent with the national prevalence reported in women of childbearing age and Brazilian children, and pointed out that the intervention measures for fighting the shortage should be extended to all pregnant and postpartum women, regardless of socioeconomic conditions and degree of nutrition knowledge, aiming to improve the health of mother and child.

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