

Serum and colostrum retinol profile in postpartum women in a Brazilian public maternity and its association with maternal and obstetric characteristics

Perfil de retinol no soro e colostro de puérperas atendidas em maternidade pública brasileira e sua associação com características maternas e obstétricas

Perfil de retinol en el suero y calostro de puérperas atendidas en maternidad pública brasileña y su asociación con características maternas y obstétricas

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ABSTRACT

Objectives: To evaluate the relationship between serum and colostrum retinol levels and maternal and obstetric characteristics of women attending a Brazilian public maternity hospital, and to establish the prevalence of vitamin A deficiency (VAD) in this population.

Methods: This cross sectional study included 97 healthy postpartum women classified according to age, nutritional status, parity, mode of delivery, newborn's weight and gestational age. Three samples of colostrum pool and a single serum sample were collected after overnight fasting in the immediate postpartum period. Retinol was analyzed by high-pressure liquid chromatography. To characterize the nutritional status of vitamin A, a cutoff for serum (30 µg/dL) and colostrum (60µg/dL) was adopted.

Results: Mean levels of serum (43µg/dL) and colostrum (60µg/dL) retinol showed adequate vitamin A nutritional status in the whole sample. High prevalence of subclinical VAD was assessed on the basis of cutoff points for serum and colostrum retinol levels: 15 and 50%, respectively. No significant correlation was found between maternal, obstetric and newborn characteristics and serum and colostrum retinol profile in the studied mothers.

Conclusions: The risk of VAD occurs in postpartum women regardless of the maternal and obstetric characteristics considered. This finding reinforces the need for special attention during prenatal care for all pregnant women in order to avoid the onset of VAD and to ensure the reduction of infant and maternal morbidity and mortality rates.

Key-words: postpartum; colostrum; serum; breast feeding; vitamin A deficiency.

RESUMO

Objetivo: Definir o estado nutricional e estabelecer a prevalência de deficiência de vitamina A (DVA) em puérperas atendidas em maternidade pública brasileira, além de avaliar a influência de características maternas e obstétricas sobre os níveis de retinol no soro e no colostro nessa população.

Métodos: Participaram do estudo de corte transversal 97 puérperas saudáveis classificadas quanto à idade, estado nutricional, paridade, via de parto, peso ao nascer e idade gestacional do recém-nascido. Três amostras de colostro e uma de soro foram coletadas em jejum no pós-parto imediato. O retinol foi analisado por cromatografia líquida de alta eficiência. Para caracterizar o estado nutricional em vitamina A,

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foi adotado ponto de corte para retinol no soro de 30µg/dL e, no colostro, de 60µg/dL.

Resultados: No grupo total de puérperas, a concentração média de 60µg/dL de retinol no colostro e 43µg/dL no soro indicou estado bioquímico adequado. No entanto, ao se avaliar individualmente, constatou-se alta prevalência de DVA subclínica no soro (15%) e no colostro (50%). Não foi verificada influência das características maternas, obstétricas e do neonato sobre os níveis de retinol no soro e no colostro das mulheres ($p>0,05$).

Conclusões: O risco de DVA pode ocorrer em gestantes/ puérperas independentemente das características maternas e obstétricas consideradas. Esse fato reforça a necessidade da atenção especial ao acompanhamento pré-natal de todas as mulheres, a fim de prevenir a instalação da DVA e garantir a redução dos índices de morbimortalidade infantil e materna.

Palavras-chave: puerpério; colostro; soro; aleitamento materno; deficiência de vitamina A.

RESUMEN

Objetivo: Definir el estado nutricional y establecer la prevalencia de deficiencia de vitamina A (DVA) en puérperas atendidas en maternidad pública brasileña, además de evaluar la influencia de características maternas y obstétricas sobre los niveles de retinol en el suero y el calostro en esta población.

Métodos: Participaron del estudio de corte transversal 97 puérperas sanas que fueron clasificadas respecto a la edad, estado nutricional, paridad, vía de parto, peso al nacimiento y edad gestacional del recién nacido. Tres muestras de calostro y una de suero fueron recogidas en ayuno en el postparto inmediato. El retinol fue analizado por cromatografía líquida de alta eficiencia. Para caracterizar el estado nutricional en vitamina A se adoptó punto de corte para retinol en el suero (30µg/dL) y en el calostro (60µg/dL).

Resultados: En el grupo total de puérperas, la concentración mediana de 60µg/dL de retinol en el calostro y 43µg/dL en el suero indicó estado bioquímico adecuado. Sin embargo, al evaluar el individuo, se constató alta prevalencia de DVA subclínica en el suero (15%) y calostro (50%). No se verificó influencia de las características maternas, obstétricas y del neonato sobre los niveles de retinol en el suero y en el calostro de las mujeres ($p>0,05$).

Conclusiones: El riesgo de DVA puede ocurrir en gestantes/ puérperas independentemente de las características maternas y obstétricas consideradas. Este hecho refuerza la necesidad de la atención especial al seguimiento prenatal de

todas las mujeres a fin de prevenir la instalación de la DVA y garantizar la reducción de los índices de morbimortalidad infantil y materna.

Palabras clave: puérperas; calostro; lactancia materna; deficiencia de vitamina A; cromatografía líquida de alta eficiencia.

Introduction

Retinol is the principal component of vitamin A and in the body its function is to aid growth, cell proliferation, cell differentiation and reproduction, to help maintain the immune system, to provide protection against night blindness and to maintain sight normality⁽¹⁾.

Vitamin A deficiency (VAD) is recognized as an important public health problem in developing countries because it has an impact on large proportions of the population and because of the severe consequences it can have for health⁽²⁾. Pregnant women are considered a high risk group and reduced blood retinol levels are often detected, especially during the third trimester of gestation⁽³⁾. Biochemical surveys available in Brazil confirm that VAD is a public health problem in many different regions, including the Northeast⁽⁴⁾.

The primary objective of VAD surveillance is to determine the magnitude, severity and global distribution of this nutritional deficiency and to achieve this objective it is necessary to define cutoff points and prevalence levels. The principal subclinical biological indicators used to define biochemical vitamin A nutritional status are serum and colostrum retinol assays. The second of these is the only way of simultaneously assessing vitamin A nutritional status in both mother and child⁽⁵⁾. A prevalence rate of the order of 10 to 20% of vitamin A deficiency among expectant mothers can be considered a moderate public health problem, while prevalence greater than 20% is indicative of a severe public health problem⁽²⁾.

Certain factors appear to modulate the levels of retinol in serum and in breastmilk, including features related to maternal and obstetric characteristics. While maternal age has a positive association with serum vitamin A levels, their association with maternal nutritional status is not yet proven⁽⁶⁻⁸⁾. Parity can also affect maternal retinol levels, suggesting that prior lactation leads to mobilization of larger retinol stocks, which is also stimulated by the greater maternal adiposity of multiparous women, with subsequent transfer to the mammary glands⁽⁹⁾. In addition to these factors, the high number of cesareans in Brazil and their increase globally means that many different studies are being conducted in order to analyze the effects of this type

of delivery on several different factors related to vitamin A deficiency. Chemical mediators, the hormones released in response to stress and the hematological immune system can all undergo changes as a result of different delivery routes⁽¹⁰⁾.

The objectives of this study were to describe the prevalence of VAD and to investigate the associations between serum and colostrum retinol levels and maternal and obstetric characteristics of women cared for at a public maternity unit in the Northeast region of Brazil.

Method

This cross-sectional cohort study was conducted between November 2009 and August 2010 with volunteer recently-delivered mothers who had been seen at a public maternity unit that is a state-level center of excellence and is located in the city of Natal, in the Brazilian state of Rio Grande do Norte. The study was approved by the Research Ethics Committee at the *Universidade Federal do Rio Grande do Norte*.

Sample size was calculated using Statcalc software (Epi-Info version 3.5.3). On the basis that the maternity unit being investigated deals with an average of 200 deliveries per month, the estimated sample size needed to achieve a 95% confidence level was 90 recently-delivered mothers. All mothers who required admission after delivery underwent the screening criteria defined for this study. The sample was selected according to the following exclusion criteria: maternal complications including pathologies (diabetes, neoplasms, gastrointestinal tract, hepatic and infectious diseases, heart diseases, syphilis, HIV positive, among others); fetal complications such as malformation; multiple births, deliveries that occurred more than 12 hours before blood was taken; use of vitamin supplements containing vitamin A or E during gestation and supplementation with a vitamin A megadose during postpartum.

The study objectives were explained to the mothers during recruitment and they gave consent to inclusion in the study and signed free and informed consent forms. The final study group therefore comprised 97 healthy recently-delivered mothers.

Data on maternal, neonatal and obstetric characteristics were harvested from prenatal care records and from a questionnaire administered by the researchers. Mothers' anthropometric nutritional status prior to becoming pregnant was determined by calculating their body mass index (BMI) using their habitual body weight prior to gestation and their height⁽¹¹⁾. Neonates' gestational ages and birth weights were classified according to the World Health Organization criteria⁽¹²⁾.

With the objective of ensuring that the data obtained were trustworthy by achieving a more homogeneous vitamin content in the biological samples, the colostrum pool method for assaying vitamin A in newborn infants was adopted rather than analyzing single samples of this type of breastmilk. In order to achieve this, 2mL of colostrum were collected for three consecutive days beginning on the first postpartum day and after 8 to 12 hours' nocturnal fasting. These samples were taken by manual expression from one breast at the start and end of the feed, in order to avoid fluctuations in fat content. On the first postpartum day, under the same fasting conditions, 5mL of blood were also taken.

Samples were taken into polypropylene tubes shielded from the light, transported in refrigerated conditions and stored at -20°C until analyses were conducted. Serum was stored under a nitrogen atmosphere at -20°C until analysis. When the first colostrum sample had been taken, before hospital discharge, mothers were given a vitamin A megadose supplement, in accordance with Brazilian Ministry of Health recommendations⁽¹³⁾.

The retinol contained in the samples was extracted using an adaptation of the method described by Ortega⁽¹⁴⁾, as follows. For 1mL of serum, 1mL of ethanol at 95% (Merck®) was used to precipitate the proteins, followed by extraction using 6mL of hexane (Merck®) and evaporation of the extract under a nitrogen atmosphere in a water bath at 37°C. When the analysis was performed, this extract was dissolved once more in 500µL of absolute HPLC grade ethanol (Vetec®) and 20µL were applied in the High Performance Liquid Chromatography machine. For retinol extraction from the colostrum samples, the above method was used with the addition of a saponification stage using 1mL of potassium hydroxide at 50% v/v (Vetec®).

The retinol concentration in the samples was determined using a Shimadzu chromatograph. This apparatus comprises an LC-20 AT Shimadzu pump coupled to an SPD-20A Shimadzu UV-VIS Detector, a Shim-pack CLC-ODS (M) 4.6mmx15cm column and a computer running LC solution software to process the data (Shimadzu Corporation®). The mobile phase used for retinol assay was methanol at 100%, in an isocratic system with a flow rate of 1mL/min and retention time of 3.2 minutes. The wavelength chosen to monitor absorbance was 325nm.

Identification and quantification of retinol in the samples were determined by comparison with the peak obtained on the chromatogram with the area of a retinol standard (Sigma®). The concentrations of the standards were confirmed using the specific extinction coefficient of retinol in absolute ethanol (ϵ 1%, 1cm=1,780 to 325nm)⁽¹⁵⁾.

Specific cutoff points were adopted for assessment of the biochemical nutritional status of the recently-delivered mothers in terms of retinol and alpha-tocopherol. The cutoffs for retinol were 30µg/dL in serum⁽¹⁶⁾ and 60µg/dL in colostrum, since these correspond to below-normal biochemical levels for expectant mothers/recently-delivered mothers⁽¹⁷⁾.

Statistical analysis was performed using Statistica 7 software (StatSoft, Inc, Tulsa, OK, USA). The results for vitamin concentrations in serum and milk were presented in means and standard deviations. The differences between means for numerical parametric variables were analyzed using Student's *t* test for dependent and independent samples. Relationships between biochemical data and maternal characteristics were investigated using Pearson's correlation coefficient. Differences were considered significant when *p*<0.05.

Results

The majority of the mothers recruited to the study (n=97) were adults (80%), multiparous (52%) and had been delivered by caesarean for the gestation being studied (54%). In terms of their pregestational nutritional status, 51% had been unhealthy, with 41% having a BMI indicative of overweight or obesity (Table 1). The majority of the neonates

Table 1 - Characteristics of the study population in terms of obstetric, maternal and neonatal variables

Characteristics	n	%
Age (years)		
15-19	21	20
> 19	73	80
Maternal nutritional status ^a		
Underweight	8	10
Healthy weight	38	49
Overweight	32	41
Parity		
Primiparous	45	48
Multiparous	48	52
Delivery		
Caesarean	53	56
Vaginal	42	44
Gestational age ^b		
Full term	75	77
Preterm	22	23
Newborn birth weight		
Low	38	40
Normal	51	54
High	6	6

^a pregestational body mass index; ^b of newborn;

(77%) were classified as full term in terms of gestational age. Although 54% had had normal birth weights, an elevated prevalence of low birth weight was observed (40%).

The mean retinol concentrations for the women in this sample were 43±11µg/dL in serum and 60±29µmol/L in colostrum. Their vitamin A nutritional status was therefore considered healthy in terms of the preestablished cutoff points for biochemical retinol levels. In the individual analyses, serum retinol assays for each of the recently-delivered mothers taken separately indicated a 15% prevalence of subclinical deficiency. Their colostrum assays revealed a higher rate of subclinical vitamin A deficiency (50%).

No significant associations were identified between maternal age, pregestational nutritional status, parity, type of delivery, neonate's gestational age and neonate's birth weight and either serum or colostrum retinol in this study population (Table 2). Furthermore, Student's *t* test did not detect any significant differences in serum or colostrum retinol for any of the maternal, obstetric or neonatal characteristics investigated.

Table 2 - Retinol levels by maternal, obstetric and neonatal characteristics and correlations between serum and colostrum levels

Variables	Retinol (µg/dL)		Pearson's Correlation ^{a,b}	
	Colostrum ^c	Serum ^c	<i>r</i>	<i>p</i>
Age (years)				
15-19	55.3±19.5	41.9±12.9	-0.04	0.88
>19	64.8±23.6	42.6± 2.2	0.05	0.64
Nutritional status ^d				
Underweight	59.0 ±17.6	38.4±6.3	-0.21	0.68
Healthy weight	58.8±23.0	42.9±13.4	0.22	0.20
Overweight	66.3±24.6	45.9±11.7	-0.01	0.97
Parity				
Primiparous	61.5±23.1	42.4±12.1	0.07	0.63
Multiparous	63.4±23.5	42.7±13.2	-0.01	0.94
Delivery				
Caesarean	63.0±20.2	40.9±12.2	-0.03	0.85
Vaginal	61.6±25.9	44.3±0.8	0.13	0.40
Gestational age ^e				
Full term	62.0±23.6	42.5±0.8	0.13	0.26
Preterm	65.9±23.1	43.2±12.0	0.23	0.29
Birth weight ^f				
Low	62.8±21.5	43.4±12.7	0.07	0.66
Normal	58.5±22.6	41.7±12.9	0.15	0.28
High	66.7±24.8	45.0±9.2	0.60	0.20

^a*r*: correlation coefficient, *p*: significance level; ^bcorrelation between serum retinol and colostrum retinol; ^cmean and standard deviation; ^dpregestational body mass index; ^egestational age; ^fweight of newborn

Discussion

The relationship between retinol levels and maternal and obstetric characteristics has been a subject of interest in several different studies^(6-9,18,19), but the results remain conflicting. An understanding of the conditions that may modulate maternal retinol levels could guide measures designed to prevent VAD. In the presence of maternal VAD, the risk of neonates suffering from the deficiency is increased and this can have a major impact on their immune systems and on the rates of infant morbidity and mortality⁽²⁾.

One of the most important factors for the health of the expectant mother and her unborn child that can be modified is maternal nutritional status and, as such, this is a critical determinant of the gestation⁽²⁰⁾. The unhealthy anthropometric status observed among the recently delivered mothers in this study together with retinol levels indicative of a public health problem demonstrate the degree to which the general status of these women is compromised and the degree to which their babies are vulnerable to VAD^(2,21). This condition demands special attention since the breastmilk secreted by mothers with inadequate vitamin A nutritional status has insufficient concentrations to build up stocks of the vitamin in the newborns' bodies⁽²²⁾, to the extent that children exclusively fed on breastmilk containing low concentrations of retinol could be suffering from subclinical VAD at 6 months if complementary foods containing sufficient quantities of vitamin A are not introduced⁽¹⁶⁾.

The picture described here is in line with the picture of subclinical VAD that has been diagnosed in Brazil over recent decades⁽⁴⁾. The elevated prevalence of VAD observed in our population is similar to rates found in Recife, PE⁽²³⁾, Rio de Janeiro⁽²⁴⁾ and São Paulo⁽²⁵⁾. Fifteen percent of the volunteers studied had serum retinol concentrations indicative of vitamin A deficiency, and 50% of the recently-delivered mothers had insufficient colostrum retinol levels. A similar result was observed among infants in Rio Grande do Norte⁽²⁶⁾ and do Rio de Janeiro⁽⁹⁾. In Rio de Janeiro the prevalence of low colostrum retinol levels was correlated with insufficient dietary vitamin A intake.

These findings corroborate the suggestion that the retinol level in milk, rather than serum retinol, is the better indicator of maternal and infant vitamin A status, particularly when considering a group of breastfeeding mothers^(27,28). The mean colostrum retinol concentration observed here was in line with what has been observed with breastfeeding mothers in industrialized nations (50 to 70µg/dL) and superior to the mean among women in non-industrialized countries (40µg/dL)⁽²⁹⁾. The figures for serum retinol observed in this study are in line with figures observed in Spain⁽¹⁴⁾ and Bangladesh⁽³⁰⁾ and in populations similar to the population studied here⁽³¹⁾ and are higher than figures for breastfeeding mothers in Africa⁽³²⁾.

Associations between maternal and obstetric characteristics and retinol levels remain contradictory. While there have been positive results⁽⁶⁾, several different studies have not detected a relationship between these factors and maternal retinol^(9,18,24,33,34).

On the basis of these findings, and considering the elevated prevalence of VAD in this study population, it is clear that there is a risk of this nutritional deficiency in expectant mothers/breastfeeding mothers irrespective of maternal and obstetric characteristics. This fact underscores the need for special attention during the prenatal care of all women in order to prevent onset of maternal VAD and to guarantee that the newborn will lay down adequate stocks in the liver, thereby contributing to reducing the rates of both infant and maternal morbidity and mortality. Armed with knowledge of this situation, it is suggested that further studies be conducted with greater numbers of samples and with a focus on investigating the influence of socioeconomic and dietary characteristics on the levels of retinol in serum and breastmilk in populations at risk of developing subclinical vitamin A deficiency.

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