

Analysis of serum retinol levels in nursing mothers with and without gestational diabetes *mellitus* attended in a public maternity hospital in Natal, Rio Grande do Norte State, Brazil

Análise da concentração de retinol sérico em lactantes com e sem diabetes *mellitus* gestacional atendidas em uma maternidade pública da Cidade de Natal, Estado do Rio Grande do Norte, Brasil

Análisis de la concentración de retinol sérico en lactantes con y sin diabetes *mellitus* gestacional atendidas en una maternidad pública de la Ciudad de Natal, Estado de Rio Grande do Norte, Brasil

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ABSTRACT

Vitamin A is essential in all stages of life, especially during pregnancy and lactation. Individuals with diabetes *mellitus* have been considered as a group at risk of showing deficiency of micronutrients and related compounds, such as vitamins A, E, and C. Accordingly, women with gestational diabetes *mellitus* (GDM) have also been identified as a group at risk of presenting low levels of vitamin A. This study aimed to compare the serum retinol of nursing women with and without GDM attended at Maternidade Escola Januário Cicco, in Natal, Rio Grande do Norte State, Brazil. Sera from 85 mothers were collected, of which 16 had DMG. The serum retinol was quantified using high performance liquid chromatography. The results were expressed by its mean and standard deviation, which presented significant differences in concentration between groups with and without GDM: the concentration levels were of $33.1 \pm 12.5 \mu\text{g/dL}$ and $41.2 \pm 11.1 \mu\text{g/dL}$, respectively. Although the mechanism responsible for changes in the levels of vitamin A in patients with diabetes has not been elucidated, it is known that the metabolism of retinol is physiologically associated with the β -cell function; consequently, when abnormalities in insulin secretion are observed, there might be changes in the concentration of retinol-binding protein and, therefore, retinol in serum. These results show the importance of monitoring the levels of this vitamin during pregnancy, especially when the mother has diabetes *mellitus*, to prevent other pathological complications in her and the infant.

Keywords: Vitamin A; Diabetes; Gestational; Vitamin A Deficiency; Insulin.

INTRODUCTION

All retinoids that have a cyclic structure of β -ionone can be called vitamin A. They include retinol (alcohol), retinal or retinaldehyde (aldehyde), retinyl esters (ester), and retinoic acid (acid)¹.

Vitamin A is essential for vision, cell growth and differentiation, and immunity^{2,3}. In addition, vitamin A is important during glycoprotein synthesis^{1,2} and reproduction^{2,3}. This vitamin is found as a preformed vitamin in foods of animal origin. Some plant foods contain precursors of vitamin A in the form of a group of compounds called carotenoids^{4,5}.

The retinyl esters are hydrolyzed in the intestine, where they release free fatty acids and retinol, the latter of which is re-esterified and incorporated into chylomicrons. Subsequently, retinol is retained in the liver and is stored in stellate cells. To be transported to other tissues, retinol combines with retinol-binding protein (RBP)¹.

During pregnancy, there is a greater requirement for vitamin A⁶, as this vitamin is essential for establishing the health of the mother and the baby. Several factors, such as age, sex, and seasonality, influence retinol serum levels⁷.

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Diabetes mellitus (DM), a heterogeneous group of metabolic disorders that have the condition of hyperglycemia in common⁸, seems to influence serum retinol levels^{7,9,10}.

Pregnancy causes some physiological changes that interfere with the metabolism of nutrients such as carbohydrates; this condition naturally predisposes pregnant women to insulin resistance. Such changes may lead to gestational diabetes mellitus (GDM)^{11,12,13}. GDM is defined as glucose intolerance that begins or is first recognized during pregnancy¹². Pregnant women who suffer from this intolerance may be considered at risk for vitamin A deficiency¹⁴. There is some evidence that abnormal secretion of RBP may occur when insulin levels are low¹⁵.

Insulin resistance during pregnancy may result from a combination of increased maternal adiposity, caloric intake, and the effects of decreased sensitivity to insulin^{8,12,16}. Such resistance leads to the preferential diversion of nutrients to the fetus and accumulation of adipose tissue in the mother¹⁶. Given that a rapid decrease in insulin resistance occurs after birth, it is suggested that the major contribution comes from the placental hormones¹².

Low levels of vitamin A in the body may increase the risk of diarrhea, respiratory infections, visual problems, and mortality¹⁷. Thus, vitamin A deficiency can lead to an increased risk of morbidity and mortality for both the mother and the child¹⁸. This study aimed to compare the serum retinol levels of nursing women with and without GDM, treated at the Januário Cicco Maternity School in Natal, Rio Grande do Norte State, Brazil.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Januário Cicco Maternity School, which is considered a tertiary referral hospital of Brazil's Unified Health System (SUS). Eighty-five mothers participated in the study. This sample of participants was obtained by convenience sampling in the period from March to November 2010 and was divided into two groups: a test group consisting of 16 mothers with GDM and a control group consisting of 69 non-diabetic mothers. The exclusion criteria were the following: women with comorbidities (cancer; gastrointestinal tract, liver, and infectious diseases; syphilis; and HIV-positive status), women with a malformed fetus, women who used vitamin supplements containing vitamin A during pregnancy, and diabetic women who did not use insulin. The women were not considered if they had given birth 12 h before the time of blood collections. Women diagnosed as diabetics were subjected to the standard diet for diabetic patients provided by the Division of Nutrition and Dietetics of the Maternity School.

Prenatal care and delivery information were collected from the medical records of each mother. Mothers consented to their participation in the study by signing a consent form approved by the Ethics and Research Committee of the Federal University of Rio Grande do Norte (protocol N°. 325/09).

The hospital nursing staff collected 5 ml of blood from each subject after they had fasted overnight for at least 8 hours. These samples were collected in polypropylene tubes previously washed with hexane to remove any traces of fat and were wrapped with aluminum foil to prevent light-induced degradation of vitamin A.

The collected blood was taken to the Laboratory of Biochemistry Research, located in the Biochemistry Department of the Biosciences Center (UFRN). The samples were centrifuged for 10 min for subsequent serum separation and removal.

Serum samples were processed according to Giuliano et al¹⁹. Hexane was used as the extraction reagent (Merck), and 2 mL was added to the serum aliquots. After each addition of hexane, the samples were agitated for 1 min and centrifuged at 4000 rpm for 10 min, and the hexane layer was removed and added to another tube. This process was performed three times.

Fifty percent of the hexane layer was subjected to a nitrogen atmosphere in a water bath at 45° C. To resuspend the extracts, 500 µL of high-purity, high-performance liquid chromatography (HPLC)-grade ethanol (Merck) was added. Subsequently, these samples were agitated for 1 min. The retinol levels in the serum were expressed in µg/dL. Maternal vitamin A deficiency was defined when retinol serum levels lower than 20 µg/dL were detected²⁰.

Retinol levels were determined by HPLC using a Shimadzu LC-10 AD Liquid Chromatograph coupled to a Shimadzu SPD-10 A UV-VIS detector and a C-R6A Chromatopac Shimadzu integrator. The chromatographic run was performed with a Shim-pack CLC-ODS (M) column (4.6 mm x 25 cm) with a mobile phase of 100% methanol and a flow rate of 1.0 mL/min. The identification and quantification of retinol in the samples analyzed were determined by comparison with the retention time and area of the respective standard. The specific extinction coefficient (E 1% 1 cm = 1850) confirmed the concentration of the standard in absolute ethanol at a wavelength of 325 nm²¹.

Retinol values were expressed as means and standard deviations. To test the differences between the means of the numerical data, the Student's t test was used. Differences were considered significant when $p < 0,05$.

RESULTS

The mean concentration of retinol found in the sera of diabetic women was 33.1 ± 12.5 µg/dL, which is considered adequate according to the reference values. For women without diabetes, the mean concentration of serum retinol was 41.2 ± 11.1 µg/dL, which is also considered adequate. There was a significant difference in the retinol levels between the groups studied ($p = 0.012$). Diabetic women presented lower serum retinol levels compared to mothers without diabetes.

None of the mothers without GDM had retinol levels below 20 µg/dL; however, 18.75% of the mothers with diabetes had a vitamin A deficiency.

DISCUSSION

A few studies that correlate serum retinol levels with GDM can be found in the literature. Basu et al²² suggest that the reduction in levels of retinol in patients with DM is due to either decreased mobilization of retinol from the liver or even to a reduced hepatic reserve of this vitamin. These authors found a significant difference between retinol serum levels of patients with and without DM, and they also observed that the levels of RBP were lower in diabetic patients.

The nutritional status of individuals with diabetes can also contribute to reduced levels of RBP¹⁰. Obese women are more susceptible to the development of GDM²³ and are therefore more prone to present lower serum retinol levels. In this study, 43.75% of mothers with GDM were obese; even considering the small number of absolute values, the results reveal a higher proportion of obese than non-obese women. This happened only in 7.25% of the mothers without diabetes.

In addition to the fact that obesity contributes to the increase in insulin resistance during pregnancy, it is important to consider the longitudinal perspective. Insulin resistance manifests in the third trimester with an approximately 50% reduction in insulin sensitivity, and in this period, there is a greater need for this hormone^{12,13}. In the United States, approximately 8% of over 4 million pregnancies are associated with diabetes; of these, 88% have GDM, which means they are not able to adequately compensate for insulin resistance¹⁶.

The analysis performed in this study was based on samples obtained immediately after birth. Even considering the rapid reduction in insulin resistance, they still reflect the final period of gestation.

There are controversies regarding the effect of insulin on plasma levels of retinol. Based on studies by Granada et al⁹, insulin-dependent diabetic patients, by making use of this hormone in the long run, tend to control the disease. However, administration of insulin does not affect the nutritional status of vitamin A. Thus, even if the participants in the study made use of insulin, this would not affect the serum levels of retinol. Conversely, Lu et al²⁴ and Tuitoek et al²⁵ stated that after treatment with insulin, vitamin A, which is stored in hepatic reserves, becomes available. This work has addressed factors that have not been well studied by investigating the relationship between retinol and insulin. This could generate new opportunities for related research topics.

CONCLUSION

Although the small number of samples does not allow for a conclusion of clinical relevance, the results indicate a trend towards lower serum retinol levels in mothers with gestational diabetes *mellitus*.

Because this pathology could be a risk factor for the development of vitamin A deficiency and could culminate in a higher risk of morbidity and mortality of both the mother and the child¹⁸, it is necessary to address the disease more broadly during pregnancy, taking into account the monitoring of insulin and the protein that carries retinol and vitamin A.

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RESUMO

A vitamina A é essencial em todos os estágios da vida, sendo ainda mais importante durante a gestação e a lactação. Indivíduos com diabetes *mellitus* têm sido considerados como um grupo em risco de apresentar deficiência em vários micronutrientes e compostos relacionados, como as vitaminas A, E e C. Em concordância, mulheres com diabetes *mellitus* gestacional (DMG) também têm sido apontadas como um grupo em risco de apresentar níveis reduzidos de vitamina A. Este estudo teve como objetivo comparar o retinol no soro de lactantes não diabéticas e com DMG atendidas na Maternidade Escola Januário Cicco, na cidade de Natal. Foram coletadas amostras de soro de 85 parturientes, das quais 16 eram portadoras de DMG. O retinol no soro foi quantificado por meio da cromatografia líquida de alta eficiência (CLAE). Os resultados foram expressos em média e desvio padrão, sendo encontrada diferença significativa entre os grupos com e sem DMG, que apresentaram concentrações de $33,1 \pm 12,5$ µg/dL e $41,2 \pm 11,1$ µg/dL, respectivamente. Embora não se tenha elucidado o mecanismo responsável pelas alterações nos níveis de vitamina A na presença do diabetes, sabe-se que o metabolismo do retinol está fisiologicamente associado à função das células-β e, por isso, em condições de anormalidade na secreção de insulina, poderá haver alterações nas concentrações de RBP sérica e, por conseguinte, de retinol. Esses resultados alertam para a necessidade do monitoramento dessa vitamina durante a gestação, principalmente na gestação acompanhada por diabetes *mellitus*, a fim de prevenir a instalação de outras complicações patológicas para a mãe e para o seu lactente.

Palavras-chave: Vitamina A; Diabetes Gestacional; Deficiência de Vitamina A; Insulina.

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RESUMEN

La vitamina A es esencial en todos los estadios de la vida, siendo todavía más importante durante la gestación y la lactación. Individuos con diabetes *mellitus* han sido considerados como un grupo en riesgo de presentar deficiencia en varios micronutrientes y compuestos relacionados, como las vitaminas A, E y C. En consecuencia, mujeres con diabetes *mellitus* gestacional (DMG) también han sido señaladas como un grupo en riesgo de presentar niveles reducidos de vitamina A. Este estudio tuvo como objetivo comparar el retinol en el suero de lactantes no diabéticas y con DMG atendidas en la Maternidade Escola Januário Cicco, en la ciudad de Natal. Fueron colectadas muestras de suero de 85 parturientes, de las cuales 16 eran portadoras de DMG. El retinol en el suero fue cuantificado por cromatografía líquida de alta eficiencia (CLAE). Los resultados fueron expresados en promedio y desvío estándar, hallándose una diferencia significativa entre los grupos con y sin DMG, que presentaron concentraciones de $33,1 \pm 12,5 \mu\text{g/dL}$ y $41,2 \pm 11,1 \mu\text{g/dL}$, respectivamente. Aunque no se haya elucidado el mecanismo responsable por las alteraciones en los niveles de vitamina A en la presencia del diabetes, se sabe que el metabolismo del retinol está fisiológicamente asociado a la función de las células- β y, por eso, en condiciones de anormalidad en la secreción de insulina, podrán haber alteraciones en las concentraciones de RBP sérica y, por consiguiente, de retinol. Esos resultados alertan para la necesidad del monitoreo de esa vitamina durante la gestación, principalmente en la gestación acompañada por diabetes *mellitus*, con el fin de prevenir la instalación de otras complicaciones patológicas para la madre y para su lactante.

Palabras clave: Vitamina A; Diabetes Gestacional; Deficiencia de Vitamina A; Insulina.



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