Placenta: a possible predictor of vitamin A deficiency

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The objective of the present study is to assess the association between vitamin A deficiency (VAD) evaluated by serum retinol concentration from the mother and umbilical cord and placental concentration of retinol and carotenoids to propose placental values representative of deficiency. Two hundred and sixty-two puerperal women and their newborns were assessed. Concentration of serum and placental retinol and carotenoids was determined by the spectrophotometric method. Receiver operating characteristic (ROC) curve analysis was performed according to two cut-off points (0.70 and 1.05 μmol/l) to represent deficiency in the placental concentration. No difference between averages of placental retinol and carotenoids was observed in the puerperal women regardless of the cut-off point used to define VAD. In relation to the newborns, a decrease (P=0.012) in placental retinol averages in individuals with VAD was observed when the 1.05 μmol/l cut-off point was adopted. In respect to the placental carotenoid averages, a decrease is observed for both the cut-off points (P=0.013 and 0.019 for 1.05 and 0.7 μmol/l, respectively). The ROC curve results point to the value of 0.80 μmol/l as representing deficiency with greater values found for sensitivity (66.7 %), specificity (41.7 %) and accuracy (65 %) when the 0.70 μmol/l cut-off point was adopted. The results of the present study show an association between the placental concentration of retinol and carotenoids with clinical VAD, suggesting the need for further studies on more severe cases of deficiency.

Placenta: Vitamin A deficiency: Newborns: Puerperal women

Vitamin A deficiency (VAD) is a public health problem of paramount relevance. Its growing prevalence has been warned since the 1990s(1–3).

Vitamin A is vital during the initial stages of life. Its role goes beyond embryonic development, tissue homeostasis, lipid metabolism and cellular differentiation and proliferation. Human placenta express factors for the nuclear transcription of retinoic acid receptors and retinoic X receptors. Modulation of these factors by retinoic acid is capable of modulating the expression of several genes such as: chorionic gonadotrophic hormone; placental lactogenic hormone; leptin; epidermal growth factor receptor; triiodothyronine; oestrogen; progesterone; cortisol; aldosterone; testosterone; vitamin D; cholesterol; fatty acids(7–9).

In 1996, the WHO underscored the need for proposed guidelines on proper selection, use and interpretation of indicators, not just to map deficiency but also to propose programmes to assess the impact of interventions to control VAD.

The placenta is the only organ composed of cells from two distinct individuals(10). So far, no studies have been done to evaluate retinol and carotenoid concentration in the placenta and its relation with the nutritional state of the mother and the child. Some authors describe the presence of receptors for the vitamin in the brush border membrane of the placenta, implying that the placenta may have a regulatory mechanism(11–13).

In this scenario, the objective of the present study was to evaluate the association between serum and placental concentration of vitamin A and to propose values of placental retinol representing VAD.

Methodology

Population and sample

The population studied was made up of low-risk puerperal women, who received antenatal care services at the maternity hospital of the Universidade Federal do Rio de Janeiro, being 262 women chosen according to the following criteria: single-child pregnancy; absence of clinically proven pathologies identified before gestation (diabetes mellitus and liver, heart

Abbreviation: VAD, vitamin A deficiency.

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Collection and analysis of placenta samples

Obtaining the placentae as well as their weighing were performed immediately postpartum after separation of the newborns\(^{(14,15)}\). Before obtaining placenta samples, the amniochorionic membrane and the umbilical cord were separated. The collection was carried out by using a surgical scalpel in a dimly lit environment\(^{(15,16)}\). Treatment, storage and transportation of the samples were carried out according to procedures described by Saunders \textit{et al.}\(^{(15)}\).

Biochemical evaluation of vitamin A nutritional status

To determine the concentration of maternal and cord blood retinol and total carotenoids, 5-ml samples of blood were collected intravenously from the puerperal women fasting for 8 h, as well as from the newborns’ umbilical cord immediately postpartum\(^{(15,17)}\). The blood samples obtained were centrifuged (3000 rpm) to separate and extract the serum and were immediately frozen at a temperature of \(-20^\circ C\) at the laboratory of the ME/UFRJ. Thereafter, all the samples were packaged in order to guarantee that the temperature was maintained during transportation to the INJC/UFRJ, where they were kept frozen until the moment the retinol and carotenoids concentration was analysed at the Institution’s Biochemical Laboratory.

Biochemical quantification

Determination of serum retinol and carotenoid concentration was performed through spectrophotometric analysis based on the Bessey \textit{et al.}\(^{(18)}\) method modified by Araujo & Flores\(^{(19)}\) and in accordance with procedures adopted by Flores \textit{et al.}\(^{(20)}\) for dosing the hepatic vitamin A. All the samples were analysed in duplicate, following the precautionary measures recommended by the International Vitamin A Consultative Group, in order to assure sample quality before analysis\(^{(16,21)}\). For a sample of nine placental portions, vitamin A concentration was also determined by HPLC\(^{(22)}\).

Cut-off points of 0.7 and 1.05 \(\mu\)mol/l were adopted to indicate VAD\(^{(23–26)}\). To indicate carotenoid insufficiency, cut-off points of < 800 \(\mu\)g/l for the puerperal women\(^{(27)}\) and < 400 \(\mu\)g/l for the newborns\(^{(27,28)}\) were adopted.

Treatment of statistics

Outlier retinol values (defined as mean \(\pm 3\) SD) were identified in two blood and seven placenta samples. All the samples originated from the blood and placenta in which these extreme values detected were excluded from the final analysis.

The Student’s \textit{t} test was used to compare means. The log transformation was used to approximate variables to the normal distribution. The paired \textit{t} test was used to compare biochemical methods. The receiver operating characteristic curve was used to establish the placental retinol and carotenoid concentration representative of their serum concentration through sensitivity and specificity evaluation for each cut-off point. The best optimal point was determined to be the one, which maximised the sensitivity and specificity values. The level of significance established was \(P<0.05\). Statistical analysis was performed using the statistical program SPSS for Windows version 15.0 (SPSS, Chicago, IL, USA).

Ethical issues

The study was carried out through an institutional accord between the Nucleus of Micronutrient Research of Josué de Castro Institute of Federal University of Rio de Janeiro (NPqM/INJC/UFRJ) and the maternity hospital (ME/UFRJ). Data collection took place after approval by the ethics commission of the said maternity school and the ethics committee of the Escola Nacional de Saúde Pública of Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Results

The puerperal participants in the study were on average of 26 (SD 5.8) years old, presented an average pre-pregnancy weight of 55.2 (SD 9) kg and total weight gain of 12.9 (SD 5.7) kg. Their newborns presented birth weights of 3.27 (SD 0.45) kg and the placentae weighed on average of 0.640 (SD 0.144) kg. Gestational duration was 39 (SD 1.6) weeks.

According to the results shown in Tables 1 and 2, a decrease in concentration in placental retinol within the VAD margins

Table 1. Placental retinol and total carotenoid averages according to maternal and newborn vitamin A nutritional state

<table>
<thead>
<tr>
<th>Cut-off point serum retinol ((\mu)mol/l)</th>
<th>Maternal</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Normal</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.05</td>
<td></td>
<td>26</td>
<td>1.37</td>
<td>2.24</td>
<td>107</td>
<td>1.95</td>
<td>3.02</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td></td>
<td>8</td>
<td>0.95</td>
<td>0.88</td>
<td>125</td>
<td>1.89</td>
<td>2.86</td>
<td></td>
</tr>
<tr>
<td>Cord</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.05</td>
<td></td>
<td>50</td>
<td>1.31</td>
<td>1.40</td>
<td>46</td>
<td>3.29</td>
<td>4.98</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td></td>
<td>26</td>
<td>1.43</td>
<td>1.74</td>
<td>70</td>
<td>2.57</td>
<td>4.18</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Placental carotenoids ((\mu)g/l)</th>
<th>Maternal</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Normal</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.05</td>
<td></td>
<td>27</td>
<td>2.0</td>
<td>1.8</td>
<td>90</td>
<td>3.4</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td></td>
<td>9</td>
<td>1.7</td>
<td>1.1</td>
<td>108</td>
<td>3.2</td>
<td>5.6</td>
<td></td>
</tr>
</tbody>
</table>

| VAD                               |          |   |      |    |         |   |      |    |
| 1.05                              |          | 44| 2.2  | 2.0 | 46      | 6.8 | 12.0 |    |
| 0.7                               |          | 23| 2.2  | 2.1 | 67      | 5.3 | 10.2 |    |

VAD, vitamin A deficiency.

Placental retinol and total carotenoids means were compared according to vitamin A status classified by serum retinol cut-off points (1.05 and 0.70 \(\mu\)mol/l) for mother and newborn. Vitamin A status was defined as VAD and normal according to each cut-off point. Placental retinol and total carotenoids means were then calculated for each group.
was observed for both the mother and the newborn, regardless of the cut-off point adopted.

Regarding carotenoids, the drop was also observed in newborns as there is a statistically significant difference between the placental carotenoid averages regardless of the cut-off point.

Analysis of the receiver operating characteristic curve was carried out for the placental concentrations of retinol according to the two cut-off points for classifying VAD both for the mother and the newborn. Values for the placental concentrations of retinol of <0.80 μmol/l were adopted as predictors of inadequate serum concentration according to values of specificity, sensitivity and the area under the curve (accuracy) (Table 3) presented. It was observed that sensitivity increases as the cut-off point for serum concentrations is lowered, in other words, as the VAD is aggravated. Additionally, regardless of the cut-off point adopted to classify serum concentration of retinol, the sensitivity and specificity results show increases in the newborn when compared with the puerperal woman. The best accuracy value (65 %) was found for the curve made from the second 0.70 μmol/l cut-off point to identify puerperal deficiency.

A receiver operating characteristic curve taken from the placental concentrations of carotenoids did not permit the adoption of any value that could represent their serum inadequacy.

No difference was found between the values obtained in retinol concentration with the spectrophotometric and with the HPLC analytical methods (P=0.318). The spectrophotometric method may be an alternative when HPLC is not available.

### Table 2. Comparison of placental retinol and total carotenoids averages after logn transformation according to maternal and newborn vitamin A nutritional state

(Mean values and standard deviations are presented as logn transformation)

<table>
<thead>
<tr>
<th>Cut-off point serum retinol (μmol/l)</th>
<th>Placental retinol (μmol/l)</th>
<th>Placental total carotenoids (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VAD</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Maternal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1·05</td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>0·25</td>
</tr>
<tr>
<td>0·7</td>
<td>8</td>
<td>0·44</td>
</tr>
<tr>
<td><strong>Cord</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1·05</td>
<td>50</td>
<td>0·18</td>
</tr>
<tr>
<td>0·7</td>
<td>26</td>
<td>0·18</td>
</tr>
</tbody>
</table>

VAD, vitamin A deficiency.

Placental retinol and total carotenoids means were compared according to vitamin A status classified by serum retinol cut-off points (1·05 and 0·70 μmol/l) for mother and newborn. Vitamin A status was defined as VAD and normal according to each cut-off point. Placental retinol and total carotenoids means were then calculated for each group.

### Table 3. Sensitivity and specificity results according to serum cut-off points for vitamin A deficiency adopting the placental cut-off point 0.80 μmol/l according to analysis of the receiver operating characteristic curve

<table>
<thead>
<tr>
<th>Serum retinol (μmol/l)</th>
<th>Puerperal (%)</th>
<th>Newborn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1·05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>59·1</td>
<td>61·2</td>
</tr>
<tr>
<td>Specificity</td>
<td>41</td>
<td>51·2</td>
</tr>
<tr>
<td>Accuracy</td>
<td>55</td>
<td>57</td>
</tr>
<tr>
<td>&lt;0·7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>66·7</td>
<td>68·0</td>
</tr>
<tr>
<td>Specificity</td>
<td>41·7</td>
<td>49·3</td>
</tr>
<tr>
<td>Accuracy</td>
<td>65</td>
<td>57</td>
</tr>
</tbody>
</table>

Discussion

The placenta is able to esterify retinoid and produce active retinoid by means of retinol, thus allowing it to produce the active metabolites it needs(6). The present study aims to evaluate the association between serum and placental concentration of vitamin A and propose a placental retinol value representing VAD.

An association between average concentrations of total placental carotenoids according to fetal nutritional states of vitamin A was found. Although the analysis of the receiver operating characteristic curve from the placental retinol concentration has shown not to predict sub-clinical deficiency, it was noted that sensitivity and specificity values increased when the cut-off point was lowered from 1·05 to 0·70 μmol/l. This fact may be interpreted as the placental vitamin A content being more related to a severer state of VAD.

In this sense, evaluation of the curve with the cut-off points at different stages of severity of the deficiency illness in question is necessary. Such an approach was not carried out in the present study, due to the fact that there were not a large enough number of grave VAD cases (according to the WHO’s cut-off points, 1996)(26) to create the curve. The same phenomenon was also noted for sensitivity and specificity values when comparing puerperal women and newborns, the results tend to be more expressive in the newborns.

In states of privation, retinol is the priority ahead of provitamin A carotenoids, being the latter converted to vitamin A as needed. It is known that the enzyme 15,15'-monooxygenase, responsible for splitting the 15,15'-monooxygenase, responsible for splitting the β-carotene molecules into two retinal molecules, is present in the fetal part of the amniotic membrane of the human placenta(6,29). This fact may account for the better association of placental concentrations with the serum concentrations of newborns, besides justifying the difficulty in finding placental concentrations of carotenoids to represent both the maternal and the newborn serum concentrations.
The placenta appears to be a possible indicator of vitamin A status for women and their newborns and could be used to
determine the prevalence of VAD. On the other hand,
during the puerperal period, the greatest transfer of vitamin A
to the neonate takes place through breastfeeding. Thus,
this organ may also contribute to the development of treatment
strategies to prevent transmission of the afore-mentioned
deficiency.

The results of the present study point to an association
between vitamin A nutritional state and the placental concentra-
tions of retinol and carotenoids. The present study using the
placenta as a marker for VAD suggests the need for further
studies to assess additional cut-off points for severe privation
and to define cut-off points for the placental concentrations.

Although spectrophotometric method is not the best for
vitamin A dosing, the present study analysed a sub-sample
with both the spectrophotometric and the HPLC methods.
Spectrophotometrics seemed to be an alternative method
when HPLC is not available. Unfortunately this analysis
could not cater for all the cases studied. So we recommend
further studies on this topic.

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and analyses; C. S. supervised the fieldwork and data
collection and participated in study design; A. R. participated
in study design. All the authors participated in manuscript
preparation. None of the authors had a personal or financial
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References

da carência de ferro e sua associação com a deficiência de
vitamina A em pré-escolares (Prevalence of iron deficien-
cy and its association with vitamin A deficiency in preschool
preschool children and women of reproductive age. J Nutr
132, 2857S–2866S.
nutrient Report. Current Progress and Trends in the Control
of Vitamin A, Iodine, and Iron Deficiencies. Ottawa: The
Micronutrient Initiative/UNICEF.
materna. Declaración conjunta OMS/FNUAP/UNICEF/Banco
Mundial (Reduction of Maternal Mortality. Joint Declaration
of OMS/FNUAP/UNICEF/World Bank). Geneva: WHO. (Classi-
ficación NLM:HB 1322.5).
5. Sommer A (1995) La carencia di vitamina A y sus consecuen-
cias. Guía práctica para la detección y el tratamiento (Vitamin A
Deficiency and Its Consequences. Practical Guide for Detection
and metabolic retinoid pathway in human amniotic membranes.
Biochem Biophys Res Commun 346, 1207–1216.
nível sérico e ingestão dietética em crianças e adolescentes
com déficit estatural de causa não hormonal (Vitamin A:
blood level and dietary intake in children and adolescents
with short stature not hormonally caused). Rev Assoc Med
Bras 48, 48–53.
of vitamin A deficiency during pregnancy on maternal and child
good, bad and variable. Sight and Life Newsletter no. 2.
10. Iyengar GV & Rapp A (2001) Human placenta as a ‘dual’ bio-
marker for monitoring fetal and maternal environment with
special reference to potentially toxic trace elements. Part I: 
physiology, function and sampling of placenta for elemental
11. Barnes AC (1951) The placental metabolism of vitamin A.
of subadequate maternal vitamin A status on placental transfer
of retinol and beta-carotene to the human fetus. Biol Neonate
69, 230–234.
The transfer of retinol from serum retinol-binding protein to
cellular retinol-binding protein is mediated by a membrane
of the placenta in relation to birth weight. J Obstet Gynaecol
Br Commonw 76, 865–872.
A re-examination of the stability of retinol in blood and serum,
and effects of standardized meal. Clin Chem 34, 
2808–2810.
de vitamina A no binômio mãe/recém-nascido em duas materni-
dades no Rio de Janeiro, Brasil (Nutritional status of vitamin A
in mother/newborn in two hospitals in Rio de Janeiro, Brazil).
Arch Latinoam Nutr 49, 318–321.
nation of vitamin A and carotene in small quantities of blood
19. Aratójo CRC & Flores H (1978) Improved spectrophotometric
distribution of vitamin A in humans and rats. Int J Vitam Nutr
Res 58, 276–280.
Biochemical Methodology for the Assessment of Vitamin A
Status. Washington: International Vitamin A Consultative
determination of retinol, tocopherol, carotenes and lycopene
in plasma by means of high-performance liquid chromatography
ness of pregnancy in rural Nepal – nutritional and health risks.
Int J Epidemiol 27, 231–237.
of vitamin A status during pregnancy. J Indian Med Assoc 98,
525–529.
vitamin A distribution curve for children aged 2–6y known to


