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[1–5].

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 placentae 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

or kidney diseases) or no use of vitamin–mineral supplementation containing vitamin A during gestation.

Collection and analysis of placenta samples

Obtaining the placentae as well as their weighing were performed immediately postpartum after separation of the newborn[14,15]. Before obtaining placentae 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 *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 $-20°C$ 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 *et al.* [18] method modified by Araujo & Flores[19] and in accordance with procedures adopted by Flores *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 µmol/l were adopted to indicate VAD[23–26]. To indicate carotenoid insufficiency,

cut-off points of < 800 µg/l for the puerperal women[27] and < 400 µg/l for the newborns[27,28] were adopted.

Treatment of statistics

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

The Student t test was used to compare means. The log transformation was used to approximate variables to the normal distribution. The paired 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

(Mean values and standard deviations)

| | Placental retinol (µmol/l) | | | | | | Placental carotenoids (µg/l) | | | | | |
| | VAD | | | Normal | | | VAD | | | Normal | | |
Cut-off point serum retinol (µmol/l)	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Maternal												
1·05	26	1·37	2·24	107	1·95	3·02	27	2·0	1·8	90	3·4	6·0
0·7	8	0·95	0·88	125	1·89	2·86	9	1·7	1·1	108	3·2	5·6
Cord												
1·05	50	1·31	1·40	46	3·29	4·98	44	2·2	2·0	46	6·8	12·0
0·7	26	1·43	1·74	70	2·57	4·18	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 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 2. Comparison of placental retinol and total carotenoid averages after log*n* transformation according to maternal and newborn vitamin A nutritional state

(Mean values and standard deviations are presented as log*n* transformation)

Cut-off point serum retinol (μmol/l)	Placental retinol (μmol/l)							Placental total carotenoids (μg/l)						
	VAD			Normal				VAD			Normal			
	n	Mean	SD	*n*	Mean	SD	*P*	*n*	Mean	SD	*n*	Mean	SD	*P*
Maternal														
1·05	26	−0·25	1·024	107	−0·13	3·02	0·668	27	−19·2	7·8	90	−19·4	12·7	0·923
0·7	8	−0·44	0·96	125	−0·14	2·86	0·521	9	−20·1	7·1	108	−19·2	12·1	0·831
Cord														
1·05	50	−0·18	1·01	46	0·22	4·98	0·141	44	−19·0	9·3	46	−12·6	12·5	0·006
0·7	26	−0·18	1·10	70	0·08	4·18	0·375	23	−19·2	10·1	67	−14·5	11·7	0·088

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.

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.

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 β-carotene 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.

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

Serum retinol (μmol/l)	Puerperal (%)	Newborn (%)
<1·05		
Sensitivity	59·1	61·2
Specificity	41	51·2
Accuracy	55	57
<0·7		
Sensitivity	66·7	68·0
Specificity	41·7	49·3
Accuracy	65	57

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 concentrations 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.

Acknowledgements

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