Serum Retinol Concentrations in Hospitalized Severe Protein-energy Malnourished Children

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Summary

Background: Vitamin A deficiency (VAD) is a major nutritional problem in many developing countries. Vitamin A status has been reported to be adversely affected in protein-energy malnutrition (PEM). Objective: to assess and compare serum retinol concentrations in hospitalized children with severe PEM and normal ones.

Methods: a prospective series of 34 malnourished and 29 normal control children, <60 months old, hospitalized at IMIP, Recife-PE were recruited for the study between August 2004 and May 2005. Serum retinol level was assayed by high performance liquid chromatography and concentrations adjusted for presence of inflammation as evidenced by levels of C-reactive protein. Blood retinol level was compared according to nutritional status (severe PEM versus normal), gender, age, maternal schooling, family income, breastfeeding practice, residence and perceived morbidity.

Results: the prevalence of low serum retinol (<0.70 μmol L⁻¹) was 41.2% in children with severe PEM and 24.1% in normal children. Serum retinol concentrations were lower in children whose mothers had low schooling (p = 0.025) and families with low per capita income (p = 0.049), regardless of their nutritional status. Serum retinol concentrations had similar distribution between children with severe PEM and normal, when adjusted for gender (p > 0.05), age (p > 0.05), maternal schooling (p > 0.05), family income (p > 0.05), breastfeeding practice (p > 0.05) and residence (p > 0.05). However, malnourished children with diarrhoea showed lower serum retinol concentrations (p = 0.021) compared to those without diarrhoea.

Conclusion: VAD was prevalent in hospitalized children with severe PEM and also among normal ones although in lesser magnitude. Intervention for prevention and control of deficiency of vitamin A are recommended in settings where diarrhea is endemic and there are unfavorable socio-economical conditions.

Introduction

Protein-energy malnutrition (PEM) is a serious health problem worldwide, and vitamin A deficiency (VAD) is one of the main micronutrient deficiencies [1]. Deficiency of vitamin A affects ~127 million children <5 years old, and 4.4 million of these suffer xerophthalmia [2]. Infectious disease is a major risk factor for developing deficiency of vitamin A because fever increases catabolism, anorexia reduces vitamin A intake and the infection itself tends to decrease intestinal absorption, impair retinol circulation and promotes urinary retinol excretion [3, 4, 5]. Since vitamin A status is assessed by measuring serum retinol concentrations, it is essential to adjust for inflammation markers [5, 6] during statistical analysis.

Studies conducted in the state of Pernambuco, northeast of Brazil [7, 8], in the 1970s, among malnourished pre-school children, showed prevalence rates of 10.2% and 18.0%, respectively, for deficiency of vitamin A, and levels of serum retinol were shown to decrease with increasing severity of malnutrition. However, there is no recent available data about serum retinol concentrations in children hospitalized with severe PEM in this ecological region.
The objective of this study was to estimate and compare serum retinol concentrations among hospitalized children with severe PEM and a normally nourished comparison groups.

Methods

Study design and cases
57 children <60 months old, hospitalized at IMIP, for severe PEM (weight/height <-3 Z scores) [9], and/or presence of pedal oedema, during the period of August 2004 and May 2005 were enrolled in the study. Children who had received mega doses of vitamin A prior to or at admission were excluded.

The comparison group comprising of children (weight/height ≥ -1 Z score) [9], without clinical oedema, hospitalized for elective surgery, were used to compare and interpret the serum retinol concentrations.

Data collection

The weight of children <2 years old was measured using a Filizola weighting scale, (model BP baby 6767-00 FILIZOLA S/A Industries, Sao Paulo, Brazil), with the maximum capacity of 15 kg, minimum of 0.125 kg and precision of 0.005 kg; length was measured using a horizontal anthropometer (Misura Per Sarti, C&C, São Paulo, Brazil) with 130 cm in length and precision of 0.1 cm. For children >2 years old, we used a Toledo electronic platform weighting scale, (model 6063866 Brazilian Toledo Weighting Industries Ltd, Sao Paulo, Brazil), maximum weight of 150 kg, minimum of 0.125 kg and precision of 0.05 kg; height was measured using the anthropometer of the weighting scale, with 2.01 m of length and precision of 0.1 cm.

A blood sample of ~3.5 ml was taken from the antecubital vein, and placed in tubes covered with aluminium paper. To separate the serum, the blood sample was centrifuged at 3000 rpm for 5 min, and divided into 2 aliquots, one for the analysis of C-reactive protein (CRP) and the other to stock in a freezer at -20°C for measurement of serum retinol at the Centre of Investigation of Micronutrients of the Federal University of Paraíba.

Serum retinol was analysed by high performance liquid chromatography (HPLC, model 305, Gilson, France), according to the technique recommended by Furr et al. [10]. Concentrations <0.70 μmol l⁻¹ (20 μg dl⁻¹) were considered low and between 0.70 and 1.05 μmol l⁻¹ (20-30 μg dl⁻¹) were taken as marginal [11].

CRP was assayed by the manual method of agglutination of particles (quantitative), using the KIT PCRTTEST (Doles Reagents and Equipments for Laboratory Ltd, Goiânia, Goiás, Brazil), consisting of a suspension of stabilized latex particles, adsorbed with anti-CRP antibodies. The cut-off point used as an indicator of inflammation was CRP >12 mg l⁻¹ [12].

Diarrhoea was defined as the occurrence of ≥3 smooth, semi-liquid or liquid stools or, at least one stool with the presence of visible blood during a period of 24 h; and, pneumonia, as the presence of cough and/or fever accompanied by tachypnoea (respiration rate >60 min⁻¹ in children <2 months old; 50 in 2-11 months old and 40 in 12-50 months old), intercostal or substernal in-drawing and flaring of alae nasi.

The study protocol was approved by the IMIP Ethical Committee for Research. The legal guardians of all the subjects signed a Free and Clarified Consent Form after being properly informed about the procedures to be followed.

Statistical analysis

Data were analysed using ANTHRO 2005 to assess nutritional status according to the WHO reference curves [9].

Categorical variables were summarized by using frequency distribution and continuous data by central tendency and dispersion measures. The comparability between severe PEM and normal children, for socio-demographic characteristics, was examined using the Chi-square test with Yates correction and, where necessary, by Fisher’s exact test. Comparison between means of serum retinol concentrations and the variables studied were undertaken using the Mann-Whitney U-test. The level of significance was 5%. Data was processed in the SPSS software for Windows, version 13.1 (SPSS Inc., Chicago, IL, USA).

Results

Of the 57 severe PEM children, 23 were excluded: 3 for previous mega dose of vitamin intake, 4 for denying the consent and 16 for levels of CRP >12 mg l⁻¹. Of the 39 normal children, 10 were excluded: 2 because of CRP >12 mg l⁻¹ and 8 for haemolysis of the blood sample.

The blood levels of retinol in the group of severe PEM children and in the group of normal ones are described in Table 1.

Only one child with severe PEM presented with possible xerophthalmia with conjunctiva and corneal xerosis, and a bilateral corneal ulcer (admission diagnosis was ectodermic dysplasia).

The distribution of serum retinol concentration among all evaluated children, regardless of their nutritional status, showed significant lower levels in children whose mothers had <8-years schooling (p=0.025) and also among those with per capita income lower than half minimum wage (p=0.049).
The comparability between the groups studied, according to the nutritional status, is presented in Table 2.

The distribution of the mean serum retinol concentrations, according to relevant mother–child variables is shown in Table 3.

Table 4 shows morbidity data.

**Discussion**

The high percentage of low serum retinol concentrations, observed in malnourished children, shows the vulnerability of this group to VAD, though, levels below 0.35 μmol l⁻¹ or overt clinical signs of xerophthalmia were not found. These findings were not unexpected, because malnourished patients suffer reduction in intestinal absorption [3], restriction of retinol circulation, immune suppression [13], leading to anorexia as well as urinary excretion of retinol [5].

Studies undertaken in India [14], South of Kivu in Congo [15] and Bangladesh [16] have reported high prevalence of low serum retinol concentrations in malnourished children.

Low levels of retinol were also found among the controls. Goetghbuer et al. [17] have described high prevalence of low blood retinol not only among malnourished African children but also in the normal ones; however in their report <0.35 μmol l⁻¹, taken as cut-off, was predominant. This differs from the present study which found a higher prevalence of marginal concentrations (0.70–1.05 μmol l⁻¹). In our study serum retinol concentrations were adjusted for markers of infection/inflammation.

The absence of children with low retinol level among those with clinical malnutrition has also been documented by previous studies conducted in Brazil in the 1970s [7, 8]. Two explanations can be offered for this. The first one is that these studies were undertaken before the implementation of the national programme for control of VAD, which advocates administration of mega doses of vitamin A to all children 6–59 months old, living in those parts of the country where VAD is prevalent. A second explanation could be that there has been recently an extension of this programme with the additional distribution of mega doses to mothers in the immediate postpartum period to guarantee its secretion in breast milk. In the present study, there has been no information of vitamin A administration to the mothers, but it was observed that the majority of the children (73.5%) had been breastfeed which could have influenced the building up of adequate hepatic store of this micronutrient in their children [18].

Comparing these findings with those in studies from Africa, where the prevalence of low blood levels of retinol was high, it is plausible that the risk of VAD in the African context is high due to endemic infections, like malaria or sepsis [19].

Adequate serum retinol levels among some malnourished subjects in our study even in the presence of diarrhoea and pulmonary infections (Table 4), needs a comment. It is known that serum retinol levels can be influenced by infection, and that malnutrition can affect the synthesis of retinol...
retinol-binding protein (RBP), both of which contribute to low serum retinol levels. Thus it is possible that the prevalence of low serum retinol levels could have been overestimated.

In acute phase of severe malnutrition serum protein levels are low [20] including those of CRP [21]. It is even more so in oedematous children [22]. Our data comprised only children with CRP level $\leq 12\, \text{mg/dl}$ indicating absence of inflammation/infection. The retinol values obtained can therefore be attributed largely to the poor nutritional status of the subjects.

The association between the low retinol levels, inadequate maternal schooling and low per capita family income demonstrates a close relationship between VAD and poor socio-economic conditions. Similar findings have been also described by Martins et al. [23], in relation to the association between VAD and low income. Brazil is considered one of the more unjust countries of the world, with great inequality of income and particularly so in the northeast region. This can explain the results of this study. Per capita family income influences several determinants of VAD, such as access to vitamin A food sources, housing conditions, education, and access to health services, particularly in rural areas. The number of years of schooling of the mothers influences acting on information, access to health services and sanitary practices. It also enhances job opportunity, and thereby contributing to the family income [24].

A similarity in the distribution of serum retinol concentrations among malnourished and normal subjects, when adjusted for sex, age, mothers’ schooling, per capita income, breastfeeding and place of residence was an unexpected result. With regard to breastfeeding, Araújo et al. [25] have observed among their series of moderate and severe xerophthalmia that almost all their patients had been weaned early.

We found an association of VAD with diarrhoea and not with respiratory infection. The role of vitamin A in the maintenance of the integrity

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### Table 3

Serum retinol concentration in severe protein-energy malnourished and normal children, hospitalized at IMIP, according to social demographic variables, Recife, Pernambuco, 2004–05

<table>
<thead>
<tr>
<th>Variables</th>
<th>Social demographic variables</th>
<th>Severe PEM</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>Age</td>
<td>$\leq 12$ months old</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>$\geq 12$ months old</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Maternal schooling</td>
<td>$&lt; 8$ years</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>$\geq 8$ years</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Per capita income</td>
<td>$&lt; R$150,000</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>$\geq R$150,000</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>Never breastfed</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>$&lt; 3$ months</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>$\geq 3$ months</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Place of residence</td>
<td>MRR</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Rural area or other states</td>
<td>23</td>
<td>15</td>
</tr>
</tbody>
</table>

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### Table 4

Serum retinol concentrations in severe protein malnourished and normal children, hospitalized at IMIP, according to morbidity, Recife, Pernambuco, 2004–05

<table>
<thead>
<tr>
<th>Variables</th>
<th>Serum retinol (μmol/L)</th>
<th>$p^{***}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>n = 34</td>
<td>Q1* Mean</td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>0.54</td>
</tr>
<tr>
<td>No</td>
<td>23</td>
<td>0.58</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>n = 34</td>
<td>Q1* Mean</td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>0.54</td>
</tr>
<tr>
<td>No</td>
<td>19</td>
<td>0.62</td>
</tr>
</tbody>
</table>

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*1st Quartile; **3rd Quartile; ***Mann-Whitney U-test; MRR=Metropolitan Region of Recife.
and differentiation of epithelial cells, and also the immune response, has been well documented. By directly affecting the anatomic and functional integrity of the gut mucosa vitamin A contributes to the increase of morbidity in infectious illnesses. Its deficiency possibly alters absorption of nutrients [3].

In relation to acute respiratory tract infection, there are observations in the literature which did not find any correlation [26, 27]. However, with regard to diarrhoea, studies seem to be in accord with our findings.

References


