

## RELATIONSHIP BETWEEN ACUTE DIARRHOEA AND LOW PLASMA LEVELS OF VITAMIN A AND RETINOL BINDING PROTEIN

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### SUMMARY

The objective of this study was to assess vitamin A status and association between acute diarrhoea and plasma levels of vitamin A through cross-sectional comparison in children.

Plasma vitamin A was measured by colorimetric method of Neeld & Pearson and RBP by radial immunodiffusion technique.

Seventy eight children (aged 18-119 months), 26 with current history of diarrhoea and 52 children as controls (outpatient from the Santa Casa de Misericórdia Hospital in metropolitan area of São Paulo City, Brazil) were studied.

Children with history of diarrhoea showed significant low levels (mean  $\pm$  s.e.) as compared to controls, vitamin A ( $15.87 \pm 1.4$   $\mu\text{g/dl}$  vs.  $21.14 \pm 1.15$   $\mu\text{g/dl}$ ,  $p < 0.007$ ) and RBP ( $1.70 \pm 0.2$   $\text{mg/dl}$  vs.  $2.52 \pm 0.11$   $\text{mg/dl}$ ).

Multivariate logistic regression adjusted by sex, age, nutritional status and mother education revealed association between diarrhoea and inadequate levels of vitamin A and RBP.

**KEYWORDS:** Diarrhoea; Vitamin A; RBP; Hypovitaminosis A.

### INTRODUCTION

Diarrhoea is the most important disease in developing countries, responsible for millions of deaths in pre-school children<sup>17</sup>. Studies have revealed synergistic relation between nutrition and infection, in this particular case vitamin A deficiency which can predispose to increased risk of acute diarrhoea and the infection aggravates vitamin A deficiency<sup>7</sup>. Association between diarrhoea and xerophthalmia, low concentration of plasma vitamin A and RBP (specific carrier protein of retinol) was reported by several studies<sup>6,15,21</sup>.

Large epidemiological prospective studies on effect of vitamin A supplementation on morbidity have not

shown protective effect on prevalence, incidence and duration of diarrhoea<sup>14,24</sup>. However, clinical trials report beneficial effects of supplementation on decrease of infant mortality<sup>13,25</sup>. Recently, in Brazilian children<sup>2</sup> a randomized double-blind, placebo-controlled trial reported that the severity of diarrhoea is decreased by vitamin A supplementation. This result is important due to the beneficial effects on morbidity and mortality always observed in populations with severe public health problems of vitamin A deficiency.

The objective of the present study was to assess vitamin A status in children through biochemical and immu-

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nological methods, besides testing association between diarrhoea and plasma levels of vitamin A and retinol binding protein in a group of Brazilian children.

## METHODS

### Subjects

Children were recruited at the Pediatric Unit of Santa Casa de Misericórdia Hospital from the central area of São Paulo, Brazil, as outpatients. The sample of children (aged between 8 months and 9 years) was part of the more large study of vitamin A status. Twenty six children attended hospital with diarrhoea (three or more loose or watery stools in the previous 24 hours); complete physical examination, biochemical, anthropometric and socioeconomic data were obtained and included in the study. Fifty two healthy children attended at the same hospital due to other problems such as minor surgeries, were used as controls. Informed consent was obtained from mother or guardian after the objectives of the study were explained. The protocol was approved by the Department of Pediatrics, Santa Casa de Misericórdia Medical School, São Paulo, Brazil.

### Procedures

Approximately 5 ml of blood were drawn between 8 and 10 a.m. from each patient, by venous puncture. Plasma was separated by centrifugation within 2 hours of collection, aliquoted and stored at -20° C for further analysis within 15 days (PENG et al.<sup>12</sup>) showed that retinol stored at -20°C is highly stable.

Plasma vitamin A and carotenoid levels were determined according to the method described by NEELD & PEARSON<sup>11</sup>. Quality control was performed in plasma kept in aliquots throughout five successive days. The daily intra-assay coefficient of variation (n = 5/day) range was 1.6% – 4.7% for vitamin A and 2.7% – 4.3% for carotenoids. Recovery from plasma enriched with three different concentrations of vitamin A (retinyl acetate, Sigma, as internal standard) was 96.8%. During the procedure plasma samples received protection against light to prevent loss of vitamin A.

Plasma RBP concentrations were determined by the conventional radial immunodiffusion technique (LC-Partigen plates, plasma retinol binding protein, Behring, kit) based on the method of MANCINI et al.<sup>10</sup>. Total plasma proteins and albumin was determined by Biuret colorimetric method.

Anthropometric measurements were made using standard procedures (WHO<sup>26</sup>). Weight for age, height for age and weight for height percentages of the reference median for each child were calculated according to the National Center for Health Statistics (NCHS) adopted in

the guidelines of the World Health Organization (WHO<sup>26</sup>).

### Statistical analysis

Data were processed and analyzed on an IBM compatible computer. Results were expressed as mean ± s.e.. Difference between mean values of groups was established using t-statistic. Differences between proportions of categorical variables were examined by the chi-square test. In order to consider that results of association between vitamin A status and diarrhoea might be influenced by age, gender and nutritional status, the effect of these variables were adjusted performing a multivariate logistic regression. Estimates of odds ratio (OR) was based on maximum likelihood techniques. In the model continuous variables were transformed in dicotomic: vitamin A (0 = vitamin A > 20 µg/dl, 1 = vitamin A < 20 µg/dl) or RBP (0 = RBP > = 1.7 mg/dl; 1 = RBP < 1.7 mg/dl) levels were independent variables and sex (0 = girls; 1 = boys), diarrhoea (0 = no; 1 = yes), age and weight for height % of reference median as independent variables. For all analysis, probability values less of 0.05 were considered significant.

## RESULTS

Seventy eight children aged between 18-119 months were studied, (x ± s.e., 49.4 ± 3.0); 26 with diarrhoea and 52 healthy controls. Forty one (52.6%) were boys and 37 (47.4%) were girls (Table 1).

Anthropometric status was assessed by weight for height as percentage of the reference median indicates that 1.9% of the control group and 7.7% of the diarrhoea group were thin.

The plasma levels (x ± s.e.) of vitamin A in the diarrhoea group and in the control group were 15.87 ± 1.4

**TABLE 1**  
Clinical and laboratorial data (mean ± s.e.) of children with acute diarrhoea and control group, São Paulo, Brazil.

	Diarrhoea (n = 26)	Control (n = 52)	P
Age (months)	43.3 ± 5.3	52.5 ± 3.7 *	0.1
Sex (m/f)	13/13	28/24	0.7
Mother education (years)	4.4 ± 0.44	4.9 ± 0.50	0.5
Albumin (gr/dl)	4.6 ± 0.1	4.6 ± 0.05	0.7
Total proteins (gr/dl)	7.2 ± 0.14	7.5 ± 0.08	0.08
Height/age (%)	98.9 ± 0.7	99.9 ± 0.8	0.4
Weight/age (%)	94.0 ± 2.2	99.3 ± 2.1	0.1
Weight/height (%)	96.0 ± 1.5	98.9 ± 1.3	0.1

TABLE 2

Odds ratios (OR) and confidence interval 95% (CI 95%) from the multivariate logistic regression with plasma levels of vitamin A and RBP as dependent variables in 78 children. São Paulo, Brazil

Independent variables	Vitamin A (< 20 µg/dl)			RBP (< 1.70 mg/dl)		
	OR	CI 95%	p	OR	CI 95%	p
Weight/height (%)	0.94	0.88-1.00	0.06	0.93	0.87-1.00	0.06
Sex (1 = m, 0 = f)	1.03	0.36-2.9	0.95	1.4	0.44-4.49	0.55
Age (years)	0.98	0.96-1.00	0.06	0.99	0.96-1.01	0.39
Mother education (years)	0.88	0.74-1.03	0.13	0.91	0.74-1.12	0.39
Diarrhoea (1 = yes, 0 = no)	5.00	1.5-16.4	0.008	3.45	1.09-10.9	0.033

µg/dl and  $21.14 \pm 1.15$  respectively; the difference was statistically significant ( $P = 0.007$ ). According to international criteria (WHO, 1994) 80.8% and 42.3% of the children had inadequate plasma vitamin A levels (< 20 µg/dl) in the diarrhoea and control group, respectively.

The means of plasma concentration of RBP  $\pm$  s.e. were  $2.52 \pm 0.1$  mg/dl in the control and  $1.70 \pm 0.2$  mg/dl in the diarrhoea group. The difference was statistically significant ( $P = 0.000$ ).

To examine the possible determinants of the diarrhoea, vitamin A and RBP association, the results of multivariate logistic regression was shown in Table 2. It included OR, confidence interval (CI) 95% and P values. Diarrhoea remained significantly associated with inadequate vitamin A levels controlled with age, sex, mother education and weight for height. When RBP levels were included as a dependent variable the same results were observed.

## DISCUSSION

This study provided evidence of association between acute diarrhoea and inadequate vitamin A status. This association remained significant after adjusted by sex, age, weight for height and mother education in a group of Brazilian children. There are several reports that revealed this potential association. In two observational studies SOMMER et al.<sup>18,19</sup> have shown a reciprocal relation between diarrhoea and vitamin A deficiency. BUYUKGEBIZ et al.<sup>3</sup> have shown that children with diarrhoea, malnutrition and infections have low levels of vitamin A as compared to controls. Also, in Congo, children with diarrhoea have vitamin A deficient levels more frequently than control group<sup>16</sup>.

We found in the present study diarrhoea as significant predictor of plasma vitamin A and RBP levels. The results of this analysis are in concordance with study in peruvian children hospitalized by diarrhoea. The associa-

tion was independent of sex, age and nutritional status<sup>15</sup>.

In many studies there is evidence of association between inadequate vitamin A status and infection. Children with pneumonia<sup>22,23</sup>, measles complicated with diarrhoea and pneumonia<sup>5,8</sup>, malaria<sup>16</sup> and other infections have low levels of vitamin A and RBP. This is attributed to various factors: low liver stores, impaired transport, increase in utilization, low absorption or short-termed food intake restriction. However, the interpretation of low levels of vitamin A in infectious diseases is very difficult; in measles and pneumonia it can be observed that low levels of circulating vitamin A probably are independent of liver reserves, because a great part of subjects showed normal levels after the acute phase of the infection. The research by FILTEAU et al.<sup>9</sup> supports the hypothesis that during acute phase response, there is a redistribution of circulating retinol. The same authors have shown that alpha-acid glycoprotein and serum amyloid, two acute phase proteins, are negatively correlated with serum retinol levels in Ghana children, including asymptomatic children.

Another mechanism could be a great increase of urinary excretion of retinol observed in adults with sepsis and pneumonia<sup>20</sup> and children with diarrhoea<sup>1</sup>. On the other hand, various double-blind placebo controlled trials were conducted to examine the effect of high doses of vitamin A on diarrhoea. The majority of studies do not show any effect on diarrhoea. However, in a recent clinical trial in Brazil<sup>2</sup>, 1240 children 6-48 months were randomized to receive large doses of vitamin A (200 000 UI) or placebo every four months, for 1 year. The study suggests that vitamin A supplementation decreased severe episodes of diarrhoea. A more dramatic reduction on incidence and duration of disease was registered by CHENG LIE et al.<sup>4</sup> in Chinese children.

Although, the available data are yet controversial and in this design study it was not possible to define the trends of association between vitamin A and diarrhoea, it

is important to consider that the prevention of diarrhoea might be an important public health intervention in the control of vitamin A deficiency in Brazilian children.

## RESUMO

### Relação entre diarreia aguda e baixos níveis plasmáticos de vitamina A e proteína carregadora de retinol (RBP)

O objetivo deste trabalho foi avaliar o estado nutricional relativo à vitamina A e a associação entre diarreia e os níveis plasmáticos de vitamina A e RBP através de um estudo transversal.

A vitamina A foi medida pelo método de Neeld & Pearson e RBP pela técnica de imunodifusão radial.

Foram estudadas 78 crianças (com idades entre 18 e 119 meses), 26 com diarreia e 52 como controles, atendidas na consulta ambulatorial do Hospital Santa Casa de Misericórdia de São Paulo, da Cidade de São Paulo.

As crianças com diarreia mostraram níveis significativamente mais baixos de vitamina A ( $15.87 \pm 1.4 \mu\text{g/dl}$  vs.  $21.14 \pm 1.15 \mu\text{g/dl}$ ,  $p < 0.007$ ) e RBP ( $1.70 \pm 0.2 \text{ mg/dl}$  vs.  $2.52 \pm 0.11 \text{ mg/dl}$ ) quando comparadas com as do grupo controle. As análises de regressão logística mostraram associação entre diarreia e níveis inadequados de vitamina A e RBP ajustados pelo sexo, idade, estado nutricional e nível de educação materna.

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