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Vitamin A deficiency and factors associated with retinol levels in public school students

Deficiência de vitamina A e aspectos associados aos níveis de retinol em estudantes de escolas públicas

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ABSTRACT

Objective

To estimate the prevalence of vitamin A deficiency and determine the socioeconomic and demographic factors associated with serum retinol levels in public school students.

Methods

This cross-sectional study included 245 students from the urban and rural areas of the city of *Teresina, Piauí,* Brazil. Socioeconomic data were collected using a form. Serum retinol level was determined by high-performance liquid chromatography and classified according to the cut-off points provided by the World Health Organization. All statistical tests had a significance level of 5%.

Results

The prevalence of vitamin A deficiency (retinol level <0.70µmol/L) was 9.8% (95%CI=7.9–10.0). The prevalences of low and acceptable retinol levels (<1.05µmol/L) were higher in students aged 12–14 years living in households

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without piped water supply (*p*>0.05). Water well or other untreated water sources were the factors most strongly associated with low retinol levels (OR=3.28; 95%CI=1.48–7.28; *p*=0.003).

Conclusion

Vitamin A deficiency was characterized as a mild public health problem in the students, indicating the need of actions that address this issue in schools and of studies with larger samples to investigate the problem at the municipal and state levels. Untreated water intake, a possible source of waterborne illnesses, contributed to lower retinol levels.

Keywords: Indicators. Students. Vitamin A. Vitamin A deficiency.

RESUMO

Objetivo

Estimar a prevalência de deficiência de vitamina A e analisar fatores socioeconômicos e demográficos associados aos níveis de retinol séricos em estudantes de escolas públicas.

Métodos

Estudo transversal conduzido com 245 estudantes da zona urbana e rural da cidade de Teresina, Piauí. Dados socioeconômicos foram coletados em formulário e o retinol sérico determinado por cromatografia líquida de alta resolução e classificado segundo os pontos de corte da Organização Mundial de Saúde. Adotou-se nível de significância de 5% para todos os testes estatísticos.

Resultados

A prevalência de deficiência de vitamina A (níveis de retinol <0,70µmol/L) foi de 9,8% (IC95%=7,9–10,0). Verificaram-se maiores prevalências de níveis de retinol baixos ou aceitáveis (<1,05µmol/L) em estudantes na faixa etária de 12–14 anos e que residiam em domicílios onde a água não era provida pela rede pública (p>0,05). Assim, ingerir água de poço ou de outra fonte não tratada foi o fator de maior impacto sobre a ocorrência de níveis mais baixos de retinol (OR=3,28; IC95%=1,48–7,28; p=0,003).

Conclusão

A deficiência de vitamina A caracterizou-se como problema de saúde pública leve entre os estudantes estudados, sinalizando a necessidade de direcionar ações que enfoquem esta problemática em escolares, bem como, de planejamento de estudos com amostras mais abrangentes para investigar o problema em âmbito municipal e estadual. A ingestão de água não tratada, possivelmente um veículo de infecção, contribuiu para menores valores de retinol.

Palavras-chave: *Indicadores. Estudantes. Vitamina A. Deficiência de vitamina A.*

INTRODUCTION

Although in decline, Vitamin A Deficiency (VAD) is still one of the great priorities regarding micronutrient deficiencies, which affect roughly 190 million children and 19.1 million pregnant women globally. VAD is classified as a moderate public health problem in 122 countries, including Brazil [1,2].

From 1991 to 2013, VAD decreased significantly in Oceania, Southeast and East Asia, Latin America, and Caribbean, but not in South Asia and Sub-Saharan Africa, places where the deficiency still prevails [3]. Brazil does not have official data yet indicating a decrease in VAD, and VAD has been considered a public health problem in the North and Northeast regions, and in some parts of the Midwest and Southeast regions, affecting mainly preschoolers, pregnant women, and puerperal women [4-7].

Some Brazilian studies conducted in the last decade found that VAD also affects a considerable proportion of school-aged children and adolescents [8-10], groups that are not classically recognized as priorities regarding vulnerability to this deficiency. Nevertheless, the findings are justified by the fact that vitamin A is strongly involved in physical growth, which can be intense in children and adolescents undergoing a growth spurt, a period that requires more vitamin A [1,2,11] Additionally, children and adolescents are susceptible to cultural, social, and environmental influences, possibly promoting inappropriate eating habits and lower intake of dietary sources of vitamins and minerals [12] and contributing to the development of important deficiency-related conditions.

Vitamin A deficiency has a negative impact on children's and adolescents' health as even subclinical deficiency levels can cause significant repercussions, compromising growth, bone health, sexual maturation, response to infections, and visual health [13] increasing morbidity, and decreasing quality of life.

Given the above and also the absence of a nationwide survey revealing the extent of VAD in Brazilian children aged more than five years and adolescents, studies are needed to investigate VAD frequency and the associated factors in these age groups. Thus, the present study aims to estimate the prevalence of VAD and analyze the socioeconomic and demographic factors associated with serum retinol levels in public school students aged eight to 14 years.

METHODS

This is a cross-sectional, descriptive, and analytical study of 245 students aged eight to 14 years attending four municipal elementary schools in *Teresina* (PI), a state in which VAD in preschoolers and pregnant adolescents is a moderate to severe public health problem [7,14,15].

Sample size was calculated by considering the 8.9% prevalence of VAD in students aged seven to 14 years from *Salvador* (BA) [10] the 32,000 students enrolled in the public schools of *Teresina* (PI) in 2011; an error margin of 5%; and a design effect of 2 as the sampling method was not random simple but cluster. The required sample size was 249 students.

All four schools were located in the Northern area of the municipality, one in the rural area and three in the urban area. First, the area (North) was randomly selected, and then the schools, also randomly selected. Male and female students aged eight to 14 years attending the selected schools were eligible for the study. Students with fever on blood collection day or students taking vitamin or mineral supplements were excluded.

Data were collected between October 2011 and March 2012. The socioeconomic and demographic data were collected by administering a form to parents and guardians. The collected data included birth date, age, sex, school location (urban/rural), parents' education levels, family income, home construction material, home water supply and source, garbage disposal system, type of sewage, and number of household members.

Five milliliters of peripheral blood were collected by venipuncture to measure serum retinol. The blood was stored in a test tube without anticoagulant and wrapped in aluminum foil to minimize the loss of vitamin A [14].

The blood samples were taken to the Laboratory of Experimental Nutrition of the Department of Nutrition of the *Universidade Federal do Piauí* (UFPI), where they were centrifuged to extract 1.0mL of serum. The serum was placed in an amber-colored Ependorff microtube, which was frozen to -80ºC and sent to the Micronutrient Investigation Center of the *Hospital Universitário Lauro Wanderley* of the *Universidade Federal da Paraíba*.

Serum retinol was quantified by the liquid chromatographer LC-10Avp (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan), consisting of a pump (SCL-10Avp), UV-VIS detector with deuterium lamp (SPD-10Avp), and manual injector controlled by the software Class-vp 6.12 SP5. Chromatographic

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separation was performed by the reversed-phase column C18 (Shimadzu LC Columm – CLC-ODS "M" 25cm; 4.6mm ID X 25cm – 5μm). The samples were prepared as follows for injection of 50μL in the High-Performance Liquid Chromatography (HPLC): thawing the serum to room temperature in the shade, pipetting 100μL of extraction solution (ethanol) and 50μL of serum in an amber-colored microtube, homogenizing and centrifuging the mixture, collecting roughly 90μL of the supernatant, and refrigerating it until HPLC injection [16].

The students' serum retinol status was classified according to the cut-off points provided by the World Health Organization [17] as follows: deficient (retinol <0.70μmol/L); acceptable (0.70 ≤ retinol <1.05μmol/L), and normal (retinol ≥1.05μmol/L). VAD was defined as serum retinol <0.70μmol/L.

The software Stata® version 12 (Stata Corp, College Station, Texas, United States) organized and analyzed the data. The variables were analyzed descriptively and presented as means, proportions, and 95% Confidence Intervals (95%CI).

The Shapiro-Wilk test assessed whether the quantitative variables had normal distribution. The Student's *t*-test compared the two means. Bivariate analysis used two categories of serum retinol: <1.05μmol/L and ≥1.05μmol/L. Pearson's Chi-square test (χ^2) or Fisher's exact test, when appropriate, tested associations between variables. The *Odds Ratio* (OR) with the *p*-values and 95%CI were used as measures of effect. Tests with $p \leq 0.05$ were considered statistically significant.

This study was approved by the Research Ethics Committee of the UFPI on July 1, 2011, under Protocol nº 0180.0.045.000-11, and all participants signed an Informed Consent Form before joining the study.

RESULTS

A total of 250 students were selected for the study, but five (2.0%) were excluded because the amount of blood drawn was inadequate for analysis. Thus, the study assessed 245 students aged eight to 14 years enrolled in four municipal public schools. Most students were female (54.7%) and aged 10 to 11 years (64.4%). Table 1 shows the socioeconomic and demographic characteristics of the sample.

The students had a mean serum retinol of 1.18μmol/L (95%CI=1.12–1.24μmol/L), which did not differ between females (1.17μmol/L; 95%CI=1.10–1.25μmol/L) and males (1.19μmol/L; 95%CI=1.10–1.29μmol/L) (*p*=0.762) (data not tabulated). Figure 1 shows the normal distribution of students' serum retinol levels by sex.

Table 2 presents the students' serum retinol levels by sex and age group. The prevalence of VAD was 9.8% (95%CI=7.9%–10.0%). A significant proportion of students (36.3%; 95%CI=26.0%–46.8%) had marginal retinol level, and 53.9% had normal retinol level (95%CI=46.4%–63.5%).

Serum retinol was significantly associated with age group (*p*=0.003). The proportion of students with marginal retinol level increased with age, and the proportion of students with normal retinol level decreased with age. However, the proportion of students with VAD did not change with age. Serum retinol was not associated with sex (*p*=0.361) (Table 2).

Table 3 shows the OR of retinol levels <1.05μmol/L by socioeconomic and demographic variables. Retinol levels <1.05μmol/L were uniformly distributed in males and females (*p*=0.280). The proportion of students with retinol level <1.05μmol/L did not vary significantly by maternal education level (OR=1.27; *p*=0.359), *per capita* income (OR=1.28; *p*=0.505), school location (OR=0.78; *p*=0.474), home construction material (OR=1.34; *p*=0.560), type of sewage (OR=0.97; *p*=0.939), or garbage disposal system (OR=0.57; *p*=0.177). However, students aged 12 to 14 years (OR=0.12; *p*=0.001) and students without piped water supply at home (OR=3.28; *p*=0.003) were more likely to have serum retinol <1.05μmol/L.

Table 1. Distribution of students by socioeconomic and demographic variables (N=245). *Teresina* (PI), Brazil, 2016.

Variables	n	%
Sex		
Male	111	45.3
Female	134	54.7
Age group (years)		
8 to 9	19	7.8
10 to 11	158	64.4
12 to 14	68	27.8
Mean (95%CI)	11.0 years (10.8–11.1 years)	
School location		
Urban	204	83.3
Rural	41	16.7
*Maternal education level**		
Up to eight years	120	49.2
>eight years	124	50.8
Mean (95%CI)	8.0 years in school (7.6–8.4 years in school)	
Per capita *income*		
Income ≤1/4 MS	93	38.0
1/4 MS <income ≤1/2 MS	119	48.5
Income >1/2 MS	33	13.5
Mean (95%CI)	204.7 *reais* (189.3–220.0 *reais*)	
Home construction material		
Masonry (finished)	143	58.4
Masonry (unfinished)	85	34.7
Other	17	6.9
*Water source**		
Piped water supply	206	84.4
Well or other	38	15.6
*Type of sewage**		
Sanitary sewer	209	85.7
Other	35	14.3
*Garbage disposal system**		
City collection service	215	88.1
Other	29	11.9

Note: Note: MS: Minimum Salary = R$622.00 (2012); *n=244.

DISCUSSION

Some studies have already been conducted on VAD in preschoolers from the urban and rural areas of the municipality of *Teresina* (PI) [14,15,18], confirming that VAD is a mild to moderate public health program in these areas.

The present study presents the first data on VAD in *Teresina's* (PI) children aged eight years or more and adolescents. Other studies conducted in municipalities or locations in the state of *Piauí* do not exist, and the present results should serve as evidence of the problem in one of the poorest regions of the country,

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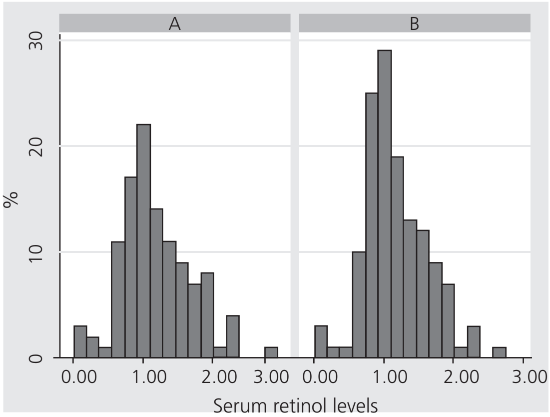

Figure 1. Distribution of serum retinol levels in the study male (A) and female (B) students. *Teresina* (PI), Brazil, 2016.

thus requiring extreme attention. In addition the data adds to the few Brazilian studies on this population group, contributing to the knowledge and understanding of the problem in population groups with possibly underestimated vulnerability to VAD.

The study vitamin A deficiency prevalence of 9.8% (95%CI=7.9%–10.0%) is characterized as a mild public health problem [1] approaching the prevalence rate that classifies the problem as a moderate public health problem (10.0%), and confirming the trend also observed in preschoolers in the region [14,15,18]. Nevertheless, that frequency is lower than the percentages reported by nearly all studies on schoolers from other Brazilian regions [8-10,19-21]. Only Mariath *et al.* [22] reported a lower VAD prevalence (2.0%) in children and adolescents monitored by a family health team in *Itajaí* (SC).

The intake of dietary sources of vitamin A grown in the region, such as moriche palm fruit (*Mauritia flexuosa*), *pequi* (*Caryocar brasiliense*), mango, pumpkin, and squash, among others, may partly justify these findings. Yet, many studies would need to investigate the intake of these fruits and vegetables by students as this population group is not very fond of fruits and vegetables [12] In addition, the use of different analytical methods to measure serum retinol and different criteria to define insufficient or deficient vitamin A status prevents comparing the VAD prevalences reported by different studies.

By publishing order, Vitolo *et al.* [19] found a VAD prevalence of 10.1% in adolescents aged 10 to 19 years attending a private school of *São Paulo* by using spectrophotometry and a cut-off point of <1.05μmol/L; 2); Ramalho *et al*. [20] found a VAD prevalence of 10.3% in students aged seven to 17 years attending a public school in *Rio de Janeiro* by using a cut-off point of <1.05μmol/L; Santos *et al.* [21] found VAD prevalence of 29.0% in children aged six to 14 years attending a rural school in *Minas Gerais* by using spectrophotometry and defining inadequate serum retinol level as <20μg/dL (<0.07μmol/L); Graebner [8] found a VAD prevalence of 33.5% in students aged

Table 2. Distribution of students' serum retinol levels by sex and age group (N=245). *Teresina* (PI), Brazil, 2016.

Serum retinol	Deficient (SR <0.70μmol/L)		Acceptable (0.70μmol/L ≤SR <1.05μmol/L)		Normal (SR ≥1.05μmol/L)		*p*
	n	%	n	%	n	%	
Sex							
Male	12	10.8	35	31.5	64	57.7	0.361*
Female	12	9.0	54	40.3	68	50.7	
Age group (years)							
8 to 9	2	10.5	1	5.3	16	84.2	
10 to 11	15	9.5	53	33.5	90	57.0	0.001**
12 to 14	7	10.3	35	51.5	26	38.2	
Total	24	9.8	89	36.3	132	53.9	

Note: *χ^2 test; **Fisher's exact test; SR: Serum Retinol.

Table 3. *Odds Ratios* (OR) of the prevalence of serum retinol level by socioeconomic and demographic characteristics. *Teresina* (PI), Brazil, 2016.

Variables	Retinol levels				$^*OR_{crude}$ (95%CI)	*p*
	<1.05μmol/L		≥1.05μmol/L			
	n	%	n	%		
Sex						
Male	47	42.3	64	57.7	1	0.280
Female	66	49.2	68	50.8	0.76 (0.46–1.26)	
Age group						
8-9 years	3	15.8	16	84.2	1	
10-11 years	68	43.0	90	57.0	0.25 (0.07–0.89)	0.032
12-14 years	42	61.8	26	38.2	0.12 (0.03–0.44)	0.001
Maternal education level						
Up to eight years	52	43.3	68	56.7	1	
>eight years	61	49.2	63	50.8	0.79 (0.48–3.31)	0.359
Per capita *income*						
≤1/2 MS	96	45.3	116	54.7	1	
>1/2 MS	17	51.5	16	48.5	0.78 (0.37–1.62)	0.505
School location						
Urban	92	45.1	112	54.9	1	
Rural	21	51.2	20	48.8	0.78 (0.40–1.53)	0.474
Home construction material						
Masonry (finished or not)	104	45.6	124	54.4	1	
Other	9	52.9	8	47.1	0.74 (0.28–2.00)	0.560
Water source						
Piped water supply	104	50.5	102	49.5	1	
Well or other	9	23.7	29	76.3	3.28 (1.48–7.28)	0.003
Type of sewage						
Sanitary sewer	97	46.4	112	53.6	1	
Other	16	45.7	19	54.3	1.03 (0.50–2.11)	0.939
Garbage disposal system						
City collection service	103	47.9	112	52.1	1	
Other	10	34.5	19	65.5	1.75 (0.78–3.93)	0.177

Note: MS: Minimum Salary: R$=622.00 (2012); $^*OR_{crude}$: Non-adjusted variables – bivariate analysis.
95%CI: 95% Confidence Interval.

five to 18 years from a rural area of the Federal District by using HPLC, which is considered the gold standard, and defining inadequate serum retinol level as <20μg/dL (<0.07μmol/L); Custódio *et al.* [9] found prevalences of 26.2% and 5.8% of serum retinol level <1.05μmol/L and <0.70μmol/L, respectively, in children aged five to 11 years by using the Relative Dose-Response method; and finally, more recently, Ribeiro-Silva *et al.* [10] found a VAD prevalence of 27.8% in children and adolescents of a public school in *Salvador* (BA), by using a cut-off point of <30g/dL or <1.05μmol/L and HPLC.

Of the biological and demographic characteristics, age influences the vitamin A status of children aged less than five years, and vitamin A level tends to decrease with age [14]. The relationship between age and retinol level in students remains unclear. Ribeiro-Silva *et al.* [10] compared students aged less and more than 10

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years from *Salvador* (BA), and found a positive association between moderate/severe VAD and age. However, the present study results do not confirm that finding as low serum retinol level was more prevalent in students aged 12 to 14 years. These students were 0.12 times more likely to have low serum retinol than students aged 8 to 9 years (*p*=0.001). More studies are needed on this subject because, from the physiological viewpoint, a growth spurt, which probably requires more vitamin A, is very complex and varies considerably between individuals, regardless of sex; additionally, the growth spurt depends on pubertal development, which is influenced by genetic and environmental factors, among others [23].

The last National Survey on the Demographics and Health of Women and Children [4] found that living in urban areas was associated with a higher prevalence of VAD. In contrast, the present study found that the proportions of students with VAD living in urban and rural areas did not differ. Even though the present study did not investigate the students' residence location, it assumed that students lived close to their schools. Other studies comparing VAD in children aged more than eight years or adolescents from urban and rural areas were not found, making this an important characteristic for future comparisons.

A growing number of studies in the scientific literature states that VAD does not depend on socioeconomic characteristics [5,24] as VAD is also a reflex of eating habits and cultural aspects that influence food choices. Most study students' families were below the poverty line, defined as families with *per capita* income between 25% and 50% of the minimum salary, according to the methodology created by Hoffmann [25]. Even so, *per capita* income was not associated with VAD, corroborating other studies [5,21].

Regarding basic sanitation, a higher proportion of low retinol levels was found in students living in homes without piped water supply, that is, piped water supply was the variable most strongly associated with low retinol levels in the study sample. This finding indicates that untreated water from wells or other sources is a source of waterborne illnesses, a determinant of low serum retinol [26].

Still, these observations must be interpreted with caution. Also, more studies are needed as the study sample is not representative of all the students aged eight to 14 years from *Teresina*. Future studies should investigate the association between water potability and serum vitamin A, and other important variables, such as food intake, for a better diagnosis of VAD in students.

CONCLUSION

The study prevalence of VAD in students aged eight to 14 years from urban and rural areas is considered a mild public health problem. Among the study socioeconomic and demographic variables, students aged more than 12 years and those whose homes had no piped water supply were more likely to have low serum retinol. These results indicate the need of society and government agencies directly or indirectly involved with public health to pay attention to this age group. Since this group is not considered at risk of vitamin A deficiency, it is not targeted by actions, policies, and programs dedicated to vitamin A prevention or treatment.

CONTRIBUTORS

ABM LIMA designed the study, collected and analyzed data, and approved the final version for publication. LS GARCÊZ analyzed data, wrote the article, and approved the final version for publication. IKF OLIVEIRA interpreted data, wrote the article, and approved the final version for publication. MM SANTOS designed the study and approved the final version for publication. SMRS PAZ and JM SILVA analyzed data, interpreted the results, and approved the final version for publication. AA PAIVA designed the study, collected and analyzed data, wrote the article, and approved the final version for publication.

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