INTRODUCTION

Vitamin A deficiency (VAD) is one of the most common and widespread nutritional deficiencies in the world and constitutes a significant public health problem.

In addition, it is one of the more common causes of potentially avoidable blindness and is responsible for the increases in morbidity-mortality due to diarrhea and respiratory causes, particularly in developing countries. In Brazil, studies have shown a high prevalence of VAD in various regions of the country.

The use of serum retinol concentration (SRC) measurements by high-performance liquid chromatography (HPLC) is recommended as a method to assess Vitamin A status of populations. In populations considered to be well nourished, however, this indicator is not a reflection of the Vitamin A liver stores and it can be normal even though liver reserves are low. Due to its strict homeostatic control, SRC is associated with Vitamin A status in the organism only in the extreme situations of deficiency and toxicity. Furthermore, it may be influenced by some factors such as infection, inflammation, and recent dietary intake. The change of SRC to a standard oral dose of Vitamin A takes into account that when Vitamin A liver reserves become low,
retinol-binding protein (RBP) accumulates in the liver before SRC decreases. Thus, when a standard dose of Vitamin A is administered, retinol binds to RBP accumulated and releases into the serum.[13-21] Relative dose response (RDR), the precursor of these tests, requires two blood samples from the same individual within a 5-h interval. The serum 30-day dose response (+S30DR) works on the same principle as the RDR test but requires the second sample within a 30–45-day interval. It has the advantage of being diagnostic and therapeutic since the oral administration of 200,000 IU retinyl palmitate is required for its application, and this dose may be sufficient for the treatment of subclinical VAD.[16]

Preliminary studies on the community where this study was conducted showed an elevated prevalence of VAD among healthy children,[21-23] but few data are available on Vitamin A status in adolescents. Thus, this study aimed to determine the prevalence of VAD in adolescents at a general pediatric outpatient clinic using the +S30DR.

**SUBJECTS AND METHODS**

This was a descriptive cross-sectional study of prevalence conducted from September 2009 to August 2010. Regarding the potential risk of teratogenic effects in the fetus,[14,24-26] the study was conducted only among males. All adolescents aged ≥10–<19 years followed up at a Primary Health Care Unit of Ribeirão Preto, Brazil, were invited to participate in the study and 100 agreed, but the ones with chronic diseases or diarrhea that might compromise their nutritional status and intestinal absorption, as well the adolescents that did not attended the second blood collection and were excluded from the final analysis, resulting in 80 participants.

The blood sample obtained after 6 h of fasting was used for the determination of serum retinol (pre-supplementation) and of C-reactive protein (CRP) and, then, an oral dose of 200,000 IU retinyl palmitate was administered. A small greasy meal with a low retinol concentration was offered after supplementation to facilitate retinol absorption. After the first collection, the adolescents were instructed to come to the unit within 30–45 days after 6 h of fasting for the collection of the second blood sample for the determination of SRC after supplementation.

Serum retinol was measured by high HPLC[27] with a model LC9 Shimadzu® (Japan) apparatus with a 25 cm × 0.46 cm C18 column (ODS), UV/Vis detector (SPD-6AV), SCL-6B controller, and C-R6A register calibrated for retinol measurement at 325 nm wavelength. The flow of the mobile phase was fixed at 2 ml/min. All-trans-retinol (Sigma® Chemical Co., St Louis, MO, USA) was used as an external standard. The detection limit of the method was 0.016 µmol/l. The +S30DR was calculated by the following formula (1): 

$$ +S30DR = \frac{(T_1 - T_0)}{T_0} \times 100. $$

Values ≥20% are indicative of low hepatic reserves of Vitamin A.[16]

Serum CRP concentrations were determined by turbidimetry to assess the possible interference of inflammatory processes with SRC and values ≥0.5 mg/dL were considered abnormal.[28-30]

An interview to obtain information about the family income, an ocular inspection was performed by a trained pediatrician for signs of xerophthalmia and anthropometric data were collected at the first visit. Z scores of body mass index were calculated to classify the nutritional status according to the criteria of the World Health Organization.[31,32]

The difference in mean retinol values before and after supplementation was analyzed by the Student’s t-test and Poisson models were adjusted to estimate the prevalence ratios of individuals with SRC ≤1.05 µmol/l before and after supplementation. A logistic regression model was used to analyze the influence of serum CRP on Vitamin A status. A second OR and 95% CI value were calculated with adjustment for CRP values. A linear regression model was adjusted to study the influence of serum CRP concentrations on pre-supplementation SRC. To determine the best sensitivity/ specificity ratio for the +S30DR of the study population and using the pre-supplementation SRC, a receiver operating characteristic (ROC) curve was constructed. The level of significance was set at 5%. The SAS software version 9.2 (SAS Institute Inc., 2008) was used.

The study was approved by the Research Ethics Committee of the University Hospital, Ribeirão Preto Medical School, University of São Paulo.

**RESULTS**

A total of 80 male adolescents participated in the study. 65.0% (52/80) were from families with monthly income up to US$ 285.00, with access to health and vaccination services, electricity, piped water, sewage system, and brick houses. 52 (65%) were classified as eutrophic, 14 (77.5%) as overweight, 10 (12.5%) as obese, and 4 (5%) as thin. 43.7% (35/80; 95% CI: 30.3–59.9%) had +S30DR tests indicative of low hepatic Vitamin A reserves. No adolescent had xerophthalmia and 2.5% (2/80) had pre-supplementation SRC ≤0.70 µmol/l. Regarding the post-supplementation SRC, only 1 (1.2%) adolescent had a value ≤0.70 µmol/l. In addition, 30.0% (24/80; 95% CI: 18.9–43.4%) of the adolescents had pre-supplementation SRC ≤1.05 µmol/l. Although 10.0% (8/80) of the adolescents still had SRC ≤1.05 µmol/l after supplementation, a significant reduction of this proportion was observed (prevalence ratio =3.0; 95% CI 1.54–5.82; P < 0.01).
The mean pre- and post-supplementation SRC were 1.30 µmol/l (SD: 0.41) and 1.54 µmol/l (SD: 0.41; P < 0.01), respectively, and the median values were 1.29 µmol/l and 1.48 µmol/l, respectively [Figure 1]. Since only two adolescents had pre-supplementation SRC ≤0.70 µmol/l, the ≤1.05 µmol/l value was used as reference for the definition of inadequate Vitamin A concentrations for the construction of the ROC curve. The cutoff point of 20% for +S30DR showed 79% sensitivity and 69% specificity, being the most adequate for the study population. A “rightward shift” was observed for the curve of frequency distribution of SRC after supplementation [Figure 2]. Serum CRP concentrations were high in 8.7% (7/80) of the adolescents, 4 of whom (11.4%–4/35) had low hepatic Vitamin A reserves by +S30DR test and 3 (6.7%–3/45) had not. However, elevated CRP concentrations did not prove to be a risk factor for low hepatic Vitamin A reserves by +S30DR test [Table 1]. In addition, there was no association between CRP values and pre-supplementation SRC (P = 0.21); thus, serum CRP concentrations did not interfere with the final value of the +S30DR. It was observed that some adolescents with elevated pre-supplementation SRC (>0.70 µmol/l) still presented +S30DR results indicative of low reserves of Vitamin A.

**DISCUSSION**

Using the +S30DR test, it was observed a high prevalence (43.7%) of low hepatic reserves of Vitamin A. There was 2.5% (2/80) of the adolescents classified as having VAD (pre-supplementation SRC ≤0.70 µmol/l)[16,33] and 30.0% (24/80) with adequate levels (pre-supplementation SRC ≤1.05 µmol/l).[15-17] A study conducted in a poor community of Rio de Janeiro (Brazil) revealed a 12.6% prevalence of SRC ≤1.05 µmol/l among adolescents of both sexes aged 10–17 years.[12] In another Brazilian study, Graebner et al.[11] observed a 31.6% prevalence of SRC ≤0.70 µmol/l among adolescents living in the rural area of Brazil. In Indonesia, a study detected an initial 41.4% prevalence of SRC ≤1.05 µmol/l among boys and a 45.1% among girls, with the proportions of serum retinol ≤0.70 µmol/l being 11.4% among boys and 7.7% among girls.[34] A study from Bangladesh on 381 adolescents from a privileged socioeconomic class detected 22% with SRC ≤1.05 µmol/l and 1.5% with concentrations ≤0.70 µmol/l.[35] Therefore, the higher prevalence detected by the +S30DR test in the present study suggests that tissue reserves of these adolescents were no longer adequate but not sufficiently depleted to influence the SRC, as also observed in a previous study on preschoolers from the same community.[21] However, the proportion of individuals with serum concentrations ≤1.05 µmol/l (30%) observed is close to those detected in the cited studies.

Despite the high sensitivity of the +S30DR, several factors support the observation that there could be a high prevalence of

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<th>CRP* (mg/dL)</th>
<th>+S30DR positive*</th>
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<tr>
<td>&lt;0.5</td>
<td>Yes n (%) 31 (42.5) No n (%) 42 (57.5) OR* (95%CI) 1.8 (0.37–8.66)</td>
</tr>
<tr>
<td>≥0.5</td>
<td>Reference 4 (57.1) 3 (42.7)</td>
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*Values ≥20%, indicative of low hepatic reserves of Vitamin A, *CRP, *odds ratio, *CI, confidence interval
VAD among the adolescents studied before supplementation with retinyl palmitate. A significant reduction of the proportion of adolescents with SRC ≤1.05 µmol/l was observed 30–45 days after supplementation, even though only 2.5% (2/80) of the adolescents had initial SRC ≤0.70 µmol/l.

Regarding the ROC curve, the cutoff point of 20% for +S30DR showed 79% sensitivity and 69% specificity, being the most adequate for the study population. In a study from the same community studied here, Ferraz et al.[21] suggested a cutoff point of 45% for the +S30DR at preschoolers. In both the study by Ferraz et al.[21] and in the present study, the prevalence of low liver reserves detected with the +S30DR was closer to the proportions of serum concentrations ≤1.05 µmol/l than to those presented by the cutoff point of 0.70 µmol/l. In view of the above considerations, these observations support the argument of some authors who propose a value of >1.05 µmol/l as being the most appropriate for SRC considered to be adequate.[3,13,36,37]

The mean SRC after supplementation was significantly higher than the pre-supplementation concentration (1.54 µmol/l and 1.30 µmol/l, respectively). A similar phenomenon was detected in Indonesia in a study in which male adolescents were found to have a significant increase in their initial SRC after weekly oral supplementation with 10,000 IU Vitamin A for 3 months.[34] In the present study, we also observed a “rightward shift” of the frequency distribution curve of the SRC after supplementation [Figure 2]. The same was also observed in preschoolers from the same community who had received an oral dose of retinyl palmitate equivalent to the one administered here.[21]

Differently of some author’s observations,[10,38-40] in the present study, there was no correlation between serum CRP and pre-supplementation SRC and high CRP values did not prove to be a risk factor for VAD. Similar findings were observed in two other studies conducted in the same community.[22,23]

Individuals were studied into a convenience sample and might not have represented the community of adolescents in the municipality of Ribeirão Preto. In addition, the relatively small number of individuals investigated may have limited the power of the study. Finally, performing the RDR, whose laboratorial logistic proceedings are difficult due to the age group, could have given more accurate information. However, by observing adolescents with pre-supplementation SRC ≤1.05 µmol/l very close to VAD prevalence detected by the +S30DR, we inferred that the latter may be helpful for adolescents. Moreover, the +S30DR can be therapeutic and can minimize the interpretation errors caused by isolated analysis of serum retinol, which can be influenced by various physiological and pathological situations.

CONCLUSION

A high prevalence of low reserves of Vitamin A was observed in a population of healthy adolescents. In addition, a dose of 200,000 IU retinyl palmitate caused a favorable change in Vitamin A status. The +S30DR proved to be a useful tool for the detection of VAD subclinical in male adolescents.

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