Giardiasis and Poor Vitamin A Status among Aboriginal School Children in Rural Malaysia

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Abstract. A cross-sectional study was carried out on 241 primary schoolchildren in Pahang, Malaysia to update their vitamin A status and to investigate the association of poor vitamin A status with their health and socioeconomic factors. All children were screened for intestinal parasitic infections. Blood samples were collected and vitamin A status was assessed. Socioeconomic data were collected by using pre-tested questionnaires. The results showed that 66 (27.4%) children had low serum retinol levels (< 0.70 μmol/L). Giardiasis and severe ascariasis were significantly associated with low serum retinol levels ($P = 0.004$ and $P = 0.018$, respectively). Logistic regression confirmed the significant association of giardiasis with low serum retinol (odds ratio = 2.7, 95% confidence interval = 1.3–5.5). In conclusion, vitamin A deficiency is still a public health problem in rural Malaysia. Vitamin A supplementation and treatment of intestinal parasitic infections should be distributed periodically to these children to improve their health and nutritional status.

INTRODUCTION

Vitamin A is an essential nutrient for cell proliferation, the immune system function, and vision.[1,2] It is also an important nutrient for gene expression and excretion of growth hormone.[3] In 1917, McCollum and Simmonds reported that vitamin A deficiency (VAD) caused development of eye lesions known as xerophthalmia in rats.[4] Vitamin A deficiency is also a well-established risk factor for childhood mortality; a vitamin A–deficient child faces a 25% higher risk of dying of a range of childhood illnesses such as measles, malaria, and diarrhea.[5]

Today, VAD is still considered a public health problem in 118 countries. Several recent studies in South Africa, northern Ethiopia, Brazil, Nepal, and Vietnam showed that the prevalence of VAD was high especially in rural areas.[6–10] According to the United Nations Children's Fund, Kenya, Benin, the Congo, Zambia, India, Afghanistan, and Myanmar still have a high prevalence of VAD, which can be as high as 70%.[5] Vitamin A deficiency is usually caused by prolonged dietary deprivation or inadequate intake as in Africa where hunger and malnutrition prevail. It is also endemic in Southeast Asia, where rice, devoid of carotene, is the staple food. Beside poverty, several studies have shown that childhood illnesses, such as measles, diarrhea, and respiratory tract infections are strong predictors of VAD.[11–13] Intestinal parasitic infections also contribute significantly to the web of causation of VAD.[14–16]

Despite great development in socioeconomic status throughout the 52 years of independence, intestinal parasitic infections and malnutrition are still public health problems in Malaysia, particularly among Aboriginal children in rural areas. Little is known about the vitamin A status of Aboriginal schoolchildren in rural Malaysia. Thus, this study was carried out to study the vitamin A status and to investigate the association of poor vitamin A status within the health and socioeconomic background of rural Aboriginal primary schoolchildren in Pahang, Malaysia.

MATERIALS AND METHODS

Study population. This study was undertaken in Pos Betau, Kuala Lipis, Pahang, Malaysia as a baseline assessment for a randomized clinical trial aimed at investigating the effects of vitamin A supplementation on intestinal parasitic reinfection among rural schoolchildren. Sekolah Kebangsaan Betau (the Betau National School), a primary school for Aboriginal children, was selected for this study. This area is considered as a remote area with a poor socioeconomic background and a high prevalence of malnutrition and parasitic infections. Most of the residents work as farmers, laborers, and rubber tappers. Some residents perform other jobs such as selling forest products. Almost all of the houses have electricity and piped water.

The school had an enrollment of 502 pupils in grades 1–6. There were 405 pupils in the target age range of 7–12 years. Of these children, 69 were absent at the time of enrollment, and 29 refused to participate. To minimize the effect of inflammation on serum retinol measurement, children with evidence of severe and chronic inflammation were excluded from the study. Thus, 15 children were excluded because they had infections with fever at the time of enrollment. Finally, 292 children were eligible and agreed voluntarily to participate in this study (universal sampling). These 292 school children provided fecal samples for examination at preliminary visits for screening for intestinal parasitic infections. Of these children, blood samples were collected from only 241 (82.5%) children who underwent a physical examination that included anthropometry. Therefore, statistical analyses for associations between variables were based on this sample size. Informed verbal consent was obtained from the participants and their parents. The protocol of this study was reviewed and approved by the Medical Ethics Committee of the University of Malaya Medical Center, Malaysia.

Demographic and socioeconomic data for study participants were collected by using a pre-tested questionnaire constructed in English and then translated into Malay, the local language. During visits to the villages, children and their parents were interviewed in their home settings by trained assistants to elicit information on the medical history, socioeconomic status, and personal hygiene practices of the study participants.

Parasitology. Fresh fecal samples were examined by using the Kato-Katz technique[17] for the presence of soil-transmitted helminthes: *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs. Egg counts, as a measure of worm burden, were also measured by using this technique, and results were recorded as eggs per gram of feces. To detect hookworm larva in light infections, the Harada-Mori fecal cultivation technique

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was used.[18] Iintensity of infection was graded as heavy, moderate, or light according to criteria proposed by the World Health Organization.[19] Scores for intensity of infections were given to each soil-transmitted helminth species (light = 1, mild = 2, and severe = 3) and mixed infections with a worm score ≥ 5 were included in the analysis. Samples were also examined for *Giardia duodenalis* trophozoites or cysts by using the trichrome staining technique.

Assessment of vitamin A status. Approximately 2–3 mL of venous blood were collected from each participant into tubes for biochemical analysis. Blood was left at room temperature to ensure clot formation. The tubes were then centrifuged at 3,000 rpm for 10 minutes to obtain serum that was stored at –20°C until further analysis. Serum retinol concentrations were determined using a reverse-phase high performance liquid chromatography (LC-10AD; Shimadzu, Kyoto, Japan) as described.[16,20] One hundred microliters of ethanol containing retinyl acetate as an internal standard was added to 100 µL of serum. Refiltered petroleum ether (RPE) (500 µL) was added and the mixture was mixed with a vortex mixer for 1 minute and centrifuged at 5,000 rpm for 10 minutes. A 150-µL sample from the refiltered petroleum ether layer was transferred into a clean test tube and evaporated. The extract was dissolved in 200 µL of ethanol. Eighty microliters of this mixture was injected into the high performance liquid chromatography apparatus. Methanol:water (95:5 [v/v]) was used as the mobile phase and all-*trans* retinol was used as the standard. For quality control, 20% of the samples were randomly selected and examined in duplicate. Serum retinol was measured in picomoles per liter.

Use of serum retinol to assess vitamin A status may be influenced by inflammation and reductions in plasma retinol, which have been described during the acute phase of a wide range of infections.[21] The design of this study did not consider the inflammatory status of the participants, but we used a cut-off value of 0.70 µmol/L (severe VAD) instead of 1.05 µmol/L (sub-clinical VAD) to reflect low serum retinol status.[16,22,23] Interpretation of results will focus on serum retinol status rather than VAD.

Data analysis. Data were analyzed by using SPSS version 11.5 (SPSS, Inc., Chicago, IL). Age of participants and serum retinol measurements showed non-Gaussian distribution and are presented as medians and interquartile ranges (IQRs). Univariate analyses were used to investigate the association between low serum retinol as the dependent variable and age, sex, educational and employment status of parents, household income, nutritional status, and intestinal parasitic infections as independent variables. $P < 0.05$ was considered statistically significant. However, to retain all possible significant associations,[24] variables that showed an association with $P \leq 0.20$ were used to develop a logistic regression model (stepwise forward).

RESULTS

General characteristics of the study population, including the socioeconomic profile, nutritional status, and intestinal parasitic infections, are shown in Table 1. A total of 292 school children who participated in this study were from 18 villages in Pos Betau, Kuala Lipis, Pahang (145 boys and 147 girls, age range = 7–12 years, median age = 10 years, IQR = 8–11 years). Approximately 34.3% of the fathers had formal education of

TABLE 1
Characteristics of 292 Aboriginal schoolchildren participating in the study, Malaysia*

Characteristics	Frequency, %
Age groups, years	
≤ 10	30.8
> 10	69.2
Female sex	51.3
Socioeconomic status	
Fathers' education (≥ 6 years)	34.3
Mothers' education (≥ 6 years)	22.4
Low household income (< 450 MYR)	81.2
Working mothers	58.1
Large family (≥ 8 members)	35.0
Supplied with piped water	87.4
Nutritional status	
Significantly underweight	37.3
Significant stunting	43.6
Parasitology	
Mixed soil-transmitted helminth infections	67.7
Ascariasis, trichuriasis, and hookworm infections	11.3
Giardiasis	17.8

* MYR = Malaysian ringgits.

at least six years. Conversely, only 22.4% of the mothers had similar formal education.

The prevalence of malnutrition was high and approximately half of the children were malnourished. The overall prevalence of trichuriasis, ascariasis, and hookworm infections were 95.5%, 67.8% and 13.4%, respectively. Approximately one-third of the children had heavy infections. The prevalence of giardiasis was 17.8%; 52 children were reported positive for *G. duodenalis*. Data on the prevalence and distribution of intestinal parasitic infections among the participants has been published.[25]

Vitamin A status. Concentrations of serum vitamin A (serum retinol) and prevalence of low serum retinol among Aboriginal schoolchildren according to age, sex, giardiasis, and ascariasis are shown in Table 2. The median concentration of serum retinol was 1.13 µmol/L (IQR = 0.69–1.41 µmol/L). It is evident that 66 (27.4%) children had serum retinol levels < 0.70 µmol/L, which is considered an indicator of clinical VAD. Moreover, 40 (16.6%) of these children had serum retinol concentrations of 0.70–1.05 µmol/L, which are considered an indicator of sub-clinical VAD. Median retinol concentrations of both sexes were almost similar, whereas children ≤ 10 years of age had a slightly higher median concentration than those > 10 years of age. The prevalence of low serum retinol (< 0.70 µmol/L) in boys and girls was 31.4% and 23.3%, respectively ($\chi^2 = 1.974$, $P = 0.160$). Prevalence of low serum retinol concentrations (0.70–1.05 µmol/L) among children > 10 years of age and those ≤ 10 years of age was 23.0% and 13.8%, respectively ($\chi^2 = 3.136$, $P = 0.077$).

The association of low serum retinol concentrations with health and socioeconomic factors was examined by using univariate and multivariate analyses. Results are shown in Table 3. The results showed that giardiasis and severe ascariasis were significantly associated with low serum retinol concentrations ($P = 0.004$ and $P = 0.018$, respectively). Conversely, there was no significant association ($P > 0.05$) between the high prevalence of low serum retinol concentrations and other explanatory factors including age > 10 years, female sex, severe trichuriasis, hookworm infection, significant underweight and stunting, low educational levels of parents, large family size, and low household income. The output of logistic regression

TABLE 2
Prevalence of low serum retinol concentration according to age, sex, giardiasis, and ascariasis among 241 Aboriginal schoolchildren, Malaysia*

| | | Prevalence of low serum retinol, % | | |
| | | Normal, > 1.05 µmol/L | Low,† 0.70–1.05 µmol/L | Low,‡ < 0.70 µmol/L |
Variable	Median retinol levels (µmol/L) (IQR)	No. (%)	No. (%)	No. (%)
Age group, years				
≤ 10 (n = 167)	1.16 (0.68–1.42)	96 (57.5)	23 (13.8)	48 (28.7)
> 10 (n = 74)	1.06 (0.70–1.40)	39 (52.7)	17 (23.0)	18 (24.3)
Sex				
M (n = 120)	1.13 (0.70–1.39)	69 (57.5)	23 (19.2)	28 (23.3)
F (n = 121)	1.12 (0.64–1.47)	66 (54.6)	17 (14.0)	38 (31.4)
Giardia infections				
Infected (n = 45)	1.02 (0.68–1.24)	20 (44.4)	3 (6.7)	22 (48.9)
Not infected (n = 196)	1.18 (0.74–1.42)	115 (58.7)	37 (18.9)	44 (22.4)
Ascaris infections				
Severe (n = 49)	0.99 (0.66–1.27)	20 (40.8)	9 (18.4)	20 (40.8)
Mild and light (n = 114)	1.19 (0.70–1.45)	63 (55.3)	22 (19.3)	29 (25.4)
Not infected (n = 78)	1.18 (0.84, 1.51)	52 (66.7)	9 (11.5)	17 (21.8)
Total (n = 241)	1.13 (0.69–1.41)	135 (56.0)	40 (16.6)	66 (27.4)

* IQR = interquartile range.
† Sub-clinical vitamin A deficiency.
‡ Severe vitamin a deficiency.

model showed that giardiasis alone (odds ratio = 2.7, 95% confidence interval = 1.3–5.5) was significantly associated with low serum retinol concentrations in these children.

DISCUSSION

Vitamin A deficiency is defined as a public health problem if ≥ 15% of a defined population has a retinol concentration < 0.70 µmol/L.[23] However, our findings showed that 27.4% had serum retinol concentrations < 0.70 µmol/L. In addition, 16.6% of them had serum retinol concentrations of 0.70–1.05 µmol/L (sub-clinical VAD). These findings were consistent with those of previous local studies conducted among Aboriginal children.[16,26]

In comparison with the neighboring countries in Southeast Asia, a higher prevalence of VAD (serum retinol concentration < 0.70 µmol/L) was reported among children in rural regions of southern Vietnam and along the southern coast of central Java in Indonesia.[10,27] During the 1990s, Indonesia showed good progress in reducing VAD but the prevalence of the transition changed after the tsunami disaster of 2004 that killed more than 200,000 persons in the territories of Ache, Nias, and Sumatra. This disaster, together with poverty, propagated the prevalence of health problems, including VAD, among children. In other parts of the world, a higher prevalence of low serum retinol concentrations were reported in Kenya,[28] South Africa,[6,29] northern Ethiopia,[7] and the Democratic Republic of the Congo.[30] This higher prevalence in other countries may be the result of the relatively better socioeconomic and nutritional status of Aboriginal children in Malaysia than for children in other parts of the developing world.

As part of our investigation of the association between intestinal parasitic infections and poor vitamin A status, we identified significant associations between giardiasis and severe ascariasis (mean eggs per gram of feces > 50,000) and low serum retinol concentrations among Aboriginal schoolchildren. Several studies reported that the absorption of vitamin A is impaired in children infected with *A. lumbricoides*[14,15] and *G. duodenalis*.[31,32]

A study on vitamin A absorption in patients with ascariasis concluded that absorption is an important contributing factor in causing clinical VAD, particularly in populations with a

TABLE 3
Association of low serum retinol with health and socioeconomic factors of 241 Aboriginal schoolchildren, Malaysia*

| | Prevalence of low serum retinol, % | | | |
Variables	Normal	Low	OR (95% CI)	P
Age > 10 years	32.0	27.3	1.2 (0.7–1.8)	0.478
Females	47.4	57.6	0.8 (0.6–1.1)	0.160
Severe ascariasis (mean epg > 50,000)	16.6	30.3	2.2 (1.1–4.2)	0.018†
Severe trichuriasis (mean epg > 10,000)	29.1	33.3	1.2 (0.7–2.2)	0.528
Hookworm infection	13.1	12.1	0.9 (0.4–2.1)	0.833
Mixed soil-transmitted helminth infection worm score ≥ 5	25.1	37.9	1.8 (0.9–3.3)	0.051
Giardiasis	14.3	30.3	2.6 (1.3–5.1)	0.004†‡
Significantly underweight	39.4	31.8	0.7 (0.4–1.3)	0.276
Significant stunting	44.6	40.9	0.8 (0.5–1.5)	0.609
Low fathers' education (< 6 years)	71.4	62.1	0.7 (0.4–1.2)	0.164
Low mothers' education (< 6 years)	81.1	77.3	0.8 (0.4–1.6)	0.502
Low household income (< 450 MYR)	79.4	83.3	1.3 (0.6–2.7)	0.495
Large family size (≥ 8 members)	30.3	22.7	0.6 (0.3–1.3)	0.245
Working mother	59.4	51.5	0.7 (0.4–1.3)	0.268

* OR = odds ratio; CI = confidence interval; epg = eggs per gram of feces; MYR = Malaysian ringgits.
† Significant association (*P* < 0.05).
‡ Confirmed as a significant association by logistic regression analysis.

marginal intake of vitamin A.[14] These authors showed that the eradication of these infections in infected children promptly led to a significant improvement in vitamin A absorption and restoration of normal vitamin A concentrations. Similarly, another study demonstrated the importance of *A. lumbricoides* as a risk factor for ocular manifestations of VAD among children in Nepal, and suggested that a reduction in the prevalence and intensity of ascariasis might reduce the incidence of xeropthalmia in the community studied.[15]

Giardiasis has been reported to cause malabsorption in children and adults and may lead to VAD. Trophozoites of *G. duodenalis* attach to the intestinal mucosa tightly by using their suckers and this attachment may result in a shortening of the absorption surfaces. Previous studies among Aboriginal children in Selangor, Malaysia, reported a higher prevalence of low serum retinol concentration and significant wasting among children infected with *G. duodealis* than in uninfected children.[16,33] Similarly, previous studies from Thailand and Mexico found that *Giardia*-infected children were more likely to be vitamin A-deficient and had lower concentrations of serum retinol than *Giardia*-free children.[31,32] Furthermore, a case of VAD phrynoderma (a rare form of follicular hyperkeratosis associated with deficiencies in vitamins A or C or essential fatty acids) has been reported to be associated with chronic giardiasis in a six-year-old boy in France.[34]

In contrast, previous reports from Brazil and Kenya found no association between intestinal parasitic infections and low serum retinol concentrations.[8,28] Moreover, a study aimed at investigating the effects of ascariasis on vitamin A in 24 children 4–10 years of age in a slum area of Dhaka City, Bangladesh, concluded that there was no association between ascariasis and malabsorption of vitamin A.[35] The authors also found no correlation between serum retinol concentration and intensities of *A. lumbricoides* infection. In addition, an oral dose of retinol (41.8 µmol/L) was given to 10 children with various severities of *A. lumbricoides* infections and only less than 1% of the supplement could be recovered in the stools collected over the following 48 hours. In addition, *A. lumbricoides* adult worms were collected and examined for their retinol content, which was found to be negative. Furthermore, previous studies among children in areas where *G. duodenalis* is hyperendemic found that *Giardia* infection has no adverse impact on their growth and vitamin A status.[8,36,37]

These inconsistencies could be caused by differences in study population and study design. The subjects of the present study were Aboriginal children residing in remote area where poverty and many health problems, including malnutrition and intestinal parasitic infections, prevail, and these conditions make it more likely that complications of infection will develop in these children. However, there is a need for further investigation to assess whether the reported association of *Giardia* infection and VAD is a causal association.

In conclusion, the findings of this study showed that VAD is still a public health problem among Aboriginal schoolchildren in rural Malaysia. There was a significant association between intestinal parasitic infections and VAD. Thus, effective control measures to reduce the prevalence of intestinal parasitic infections should be considered, together with vitamin A supplementation, in public health strategies for the control of VAD among these children to improve their health and nutritional status.

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