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Endemic Goiter in Semirom; There Is No Difference in Vitamin A Status between Goitrous and Nongoitrous Children

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Summary Background: Despite long-standing iodine supplementation in Iran, the prevalence of goiter remains high in some areas. This suggests other nutritional deficiencies may be considered as responsible factors for goiter persistence. In the present study we investigated the possible role of vitamin A deficiency (VAD) and low vitamin A status in the etiology of endemic goiter in Semirom, Iran. Materials and Methods: In this cross-sectional study, 1,828 students from 108 primary schools of urban and rural areas of Semirom were selected by multistage random cluster sampling. Thyroid size was estimated in each child by inspection and palpation. Urinary iodine concentration (UIC) and serum retinol (SR) were measured. Results: Overall, 36.7% of schoolchildren had goiter. The median UIC was 18.5 μg/dL. The mean±SD of SR in goitrous and nongoitrous children was 38.84± 10.98 and 39.17±10.85 μg/dL respectively (p=0.82). There were two children with VAD (SR less than 20 μg/dL); one in the goitrous and one in the nongoitrous group. The prevalence of subjects with low vitamin A status (SR less than 30 μg/dL) in the goitrous and nongoitrous groups was 26.2 and 21.5% respectively (p=0.42). Conclusion: Goiter is still a public health problem in this region. Iodine deficiency, VAD or low vitamin A status is not among the contributors of goiter persistence in schoolchildren of Semirom. The role of other micronutrient deficiencies or goitrogens should be investigated.

Key Words goiter, iodine deficiency, vitamin A deficiency, low vitamin A status

Iodine deficiency (ID) is recognized as the major preventable public health problem worldwide. It is estimated that 750 million people worldwide are at risk of iodine deficiency disorders (IDD) (*1*). IDD can be presented with a wide variety of clinical manifestations ranging from congenital anomalies, cretinism, deaf mutism, psychomotor defects and severe hypothyroidism to goiter (*2*). Vitamin A deficiency (VAD) is a major public health problem too; those most vulnerable include preschool children and pregnant women in low-income countries. In children, VAD is the leading cause of preventable visual impairment and blindness (*3*). In a meta-analysis in 2002, it was estimated that there were 127.2 million vitamin A (VA) deficient preschool-aged children, representing 25% of preschool-aged children in high-risk regions of the developing world (*4*). These deficiencies often coexist in children in developing countries (*5*).

In animals, VAD has multiple effects on thyroid metabolism (*6*). In the thyroid, VAD decreases thyroidal iodine uptake and iodine incorporation into thyroglobulin (Tg) and increases thyroid size (*7–11*); in the periphery, VAD increases circulating thyroid hormone concentrations (*12*); and in the pituitary, VA status modulates thyrotropin (TSH) production by retinoid X receptor (RXR)-mediated expression of pituitary TSH-β mRNA (*12–18*), and VAD in rats increases pituitary TSH-β mRNA, TSH, and circulating thyroid hormone (*12*). High-dose VA supplementation (VAS) (without iodine supplementation (IS)) in rats with concurrent VAD and ID reduces pituitary TSH-β mRNA expression, circulating TSH, and thyroid weight (*19*).

Although VAD and IDD are common in many developing countries, there are few human data on their potential interaction in endemic regions (*5*). In a recent randomized, double-blind 2×2 intervention trial in South African children with mild to moderate VAD and ID it was suggested that high-dose VAS in a population could modify indicators of ID, such as thyroglobulin and goiter, independent of a change in iodine nutrition. It was also concluded that through suppression of transcription of the pituitary TSH-β gene, VAS might decrease excess TSH stimulation of the thyroid and thereby reduce the risk of goiter (*20*).

Endemic goiter was present in most parts of Iran (*21*) and ID was considered a contributing factor for endemic goiter in this country (*22*). Iran's National Committee for Control of IDD was initiated in 1989 by the order of the Minister of Health and Medical Educa-

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tion. The production and distribution of iodized salt, with 40 mg of potassium iodide per kg of sodium chloride began and the education of policymakers, health personnel and the public initiated in 1990. However, a rapid survey of iodized salt consumption showed that less than 50% of the population consumed iodized salt in 1993 with a mean urinary iodine of 5.0 to 8.2 μg/dL. Therefore, the first law requiring the mandatory iodization of all salts for household use was proclaimed in 1994 (*23*). Semirom is a mountainous region in the central area of Iran, where goiter was hyperendemic with a prevalence of about 89.5% estimated in 1994 (*24*). In 1993 all citizens of Semirom were given a single-dose injection of 480 mg iodized oil intramuscularly. Because the prevalence of goiter has not decreased as expected, years after iodine injection and salt iodization, an important question emerged: which factors other than iodine deficiency are responsible for the persistence of endemic goiter in this area (*25*)?

The present study was carried out to estimate the goiter prevalence and iodine status and to investigate the role of VAD as a possible contributor of endemic goiter in Semirom schoolchildren.

MATERIALS AND METHODS

This was a cross-sectional study performed on schoolchildren of Semirom in 2003. Subjects were enrolled with a multistage cluster random sampling. The exclusion criteria were: subjects with a history of exposure to radioactive iodine, thyroid surgery, or significant underlying disease such as cardiopulmonary, liver or renal problems based on available medical records and interviews with parents and teachers. Goiter grading was performed by two endocrinologists according to WHO/UNICEF/ICCIDD classification (*1*).

Weight and standing height were measured. Height was recorded to the nearest 0.5 cm and weight was recorded to the nearest 100 g. Body mass index (BMI) was calculated using the following formula: BMI= weight (kg)/height $(m)^2$. Body surface area (BSA) was calculated by the formula: weight $(kg)^{0.425}\times$height $(cm)^{0.725}\times 71.84\times 10^{-4}$.

The blood samples were transported on dry ice to the reference laboratory of the Isfahan Endocrine and Metabolism Research Center. The samples were stored at $-70^{\circ}C$ until analysis. All urine and blood assays were performed within a median of 26 h of sampling. The same person performed each assay using the same method. Urine iodine concentration (UIC) was measured by the digestion method based on a modification of the Sandell-Kolthoff reaction (*1, 26*) (intra-assay CV 1.2% and inter-assay CV 2.2%). SR was measured by HPLC (*27*). VAD was defined as an SR less than 20 μg/dL (*28*) and an SR concentration less than 30 μg/dL indicated low VA status (*4*).

Serum T4 concentrations were measured with radioimmunoassay (RIA) by Iran Kavoshyar kits (Intra-assay CV 4.7% and inter-assay CV 4.9%). The normal range for T4 level was 4.5–12 μg/dL. Serum TSH concentration was determined with immunoradiometric assay (IRMA) by Iran Kavoshyar kits (Intra-assay CV 1.5% and inter-assay CV 1.9%). The normal range for TSH level was 0.3–3.9 mU/L.

Serum iron was measured by photometric assay (intra-assay CV 2.2% and inter-assay CV 2.9%) and transferrin by immunoturbidimetry (intra-assay CV 2.7% and inter-assay CV 3.1%), both with an automated analyzer (Liasys, Italy). Serum ferritin was measured with IRMA by Iran Kavoshyar kits (intra-assay CV 5.9% and inter-assay CV 5.5%).

Anti Tg and anti TPO were measured by Rapid ELISA (Genesis Diagnosis Co.). Intra and inter-assay CV for Anti-Tg Ab were $<12\%$ and for TPO Ab were 7.1 and 5.2%, respectively. Quantitative variables are presented as mean$\pm$SD. An independent sample *t*-test was used to compare normally distributed data in different groups. Prevalence of low VA status between goitrous and nongoitrous children was compared by the Chi-square test. A p value less than 0.05 was considered statistically significant. All analyses were performed using SPSS version 15.

Written consent was obtained from all children's parents who were informed about the study. The study was approved by the ethics committee of the Isfahan Endocrine and Metabolism Research Center of Isfahan University of Medical Sciences.

RESULTS

One thousand eight hundred and twenty-eight schoolchildren (879 from urban and 949 from rural regions) were enrolled in this study with a male-to-female ratio of 1.17. Their age ranged from 7 to 13 y. The mean age$\pm$SD was 9.31$\pm$1.05 for girls and 9.35$\pm$1.00 for boys. Overall 36.7% of subjects were classified as goitrous (Table 1). Goiter prevalence among girls was 38.6% while 35.0% of boys were goitrous (p=0.06).

The mean$\pm$SD and median UIC was 19.3$\pm$9.1 and 18.5 μg/dL respectively. Six and four tenth percent of total samples had iodine excretion levels below 10 μg/dL and 3.2% had iodine levels below 5 μg/dL. UIC in goitrous and nongoitrous children was 19.46$\pm$7.57 μg/dL and 19.31$\pm$ 10.14 μg/dL respectively (p=0.9). The mean$\pm$SD of UIC in goitrous and nongoitrous boys was 30.78$\pm$ 6.44 μg/dL and 20.4$\pm$9.34 μg/dL respectively (p=0.7). UIC in goitrous and nongoitrous girls was 18.69$\pm$8.15 μg/dL and 18.54$\pm$10.98 μg/dL respectively (p= 0.9).

SR was measured in 107 grade 2 goitrous (54 boys and 53 girls) and 107 nongoitrous children (59 boys

Table 1. Thyroid size determined by inspection and palpation in schoolchildren of Semirom, Iran.

	n	Thyroid size		
		Grade 0	Grade 1	Grade 2
Boys	989	65%	29.2%	5.8%
Girls	839	61.4%	31.7%	6.9%
All	1,828	63.3%	30.4%	6.3%

Table 2. Serum levels of different variables in children with and without low VA status in Semirom, Iran.

	Low VA status (n=51)	Sufficient VA status (n=163)	p
TSH (mU/L)	2.67±1.55	2.74±2.42	0.85
T4 (μg/dL)	9.01±1.74	8.84±1.51	0.50
Iron (μg/dL)	67.58±27.87	80.86±37.35	<0.01
Transferrin (μg/dL)	336.69±38.61	345.46±49.86	0.25
Ferritin (ng/mL)	34.18±20.94	30.69±17.16	0.23
Anti TPO (IU/mL)	116.78±560.15	36.59±273.13	0.32
Anti Tg (IU/mL)	59.39±302.13	98.68±585.54	0.65
UIC (μg/dL)	22.17±8.98	19.06±9.77	0.28

and 48 girls) as control group. They were randomly selected and were matched according to age. Although the mean±SD of SR in goitrous children was lower than in the control group (38.84±10.98 vs. 39.17± 10.85 μg/dL) the difference was not statistically significant (p=0.82). The mean±SD of SR in goitrous and nongoitrous boys was 38.30±10.59 μg/dL and 38.79± 12.21 μg/dL respectively (p=0.82). The mean±SD of SR in goitrous and nongoitrous girls was 39.38±11.45 μg/dL and 39.65±9.01 μg/dL respectively (p=0.90). In the 214 children in which SR was measured, only two children had VAD; one in the goitrous group and one in the control group. In the goitrous and nongoitrous groups there were 28 (26.2%) and 23 (21.5%) children with low VA status respectively (p=0.42). The prevalence of subjects with low VA status in goitrous and nongoitrous boys was 25.9 and 25.4% respectively (p=0.95). The prevalence of subjects with low VA status in goitrous and nongoitrous girls was not statistically significant, either (26.4% vs. 16.7%, p=0.24). In children in which SR was measured there was no child with overt hypothyroidism and 14.2% of children had subclinical hypothyroidism. There was no statistically significant difference between the mean of SR in euthyroid and hypothyroid subjects (39.09±10.81 vs. 38.09±11.63 μg/dL, p=0.65). Whilst 14.6% of children with low VA status were subclinically hypothyroid, 14.1% of children with sufficient VA had subclinical hypothyroidism (p=0.93).

The mean serum TSH, T4, iron, transferrin, ferritin, anti TPO, anti Tg, and UIC in subjects with and without low VA status were not significantly different (Table 2). The prevalence of subclinical hypothyroidism in children with low and sufficient VA status was 15.2 and 18.5% respectively (p=0.39).

SR level was correlated with serum iron level (r=0.29, p<0.001). There wasn't any significant correlation between serum VA level and age, BMI, BSA, TSH, T4, transferrin, ferritin, or UIC levels.

There was no statistically significant difference in weight, height, or BMI between goitrous and nongoitrous subjects.

DISCUSSION

According to the present study goiter prevalence in Semirom has decreased from about 89.5% in 1994 (*24*) to 36.7% in 2003. This implies ID has been the most important cause of endemic goiter and also shows the effective role of the legislation and salt iodization in treating goiter. However goiter is still endemic in this iodine replenished area and a severe public health problem in this region according to WHO/UNICEF/ICCIDD recommended criteria (*1*).

According to WHO/UNICEF/ICCIDD recommended criteria, the indicator of ID elimination is a median value for UIC of 10 μg/dL, and UIC should not be below 5 μg/L in more than 20% of samples (*1*). In the studied population the median UIC was 18.5 μg/dL and 3.2% of the population had UIC below 5 μg/dL. It means there is no biochemical ID or no inadequacy in iodine intake of the overall population.

It has been shown that unknown goitrogens (*29*), autoimmunity (*30*), or other micronutrient deficiencies such as selenium deficiency (*31*) or iron deficiency (*32*) can also contribute to the persistence of endemic goiter within a population. Previously we showed that autoimmunity may play a role in the residual goiter in Semirom (*30*) but the role of iron deficiency and selenium deficiency in the etiology of endemic goiter in this region have been excluded (*25*, *33*).

VA deficiency remains a leading public health problem in the developing world, with its health consequences most apparent and severe among infants, young children, and women of reproductive age (*34*). VAD has multiple effects on thyroid metabolism that may be dependent on iodine status (*19*). For instance it was shown that VA-deficient rats had significantly higher serum T4, TSH, and T3 levels than were found in VA-sufficient controls (*12*). It was also found that VA and retinoids could suppress serum TSH levels and decrease TSH-β subunit gene promoter activity (*12*, *35*). These findings are different from our study, in which there was not any significant difference between T4 and TSH levels in children with and without low VA status. There are few human data on the potential interaction of VAD and ID in endemic regions (5). These studies sometimes had different results from the animal studies. In contrast to our study, in a cross-sectional study in Morocco, VAD in children with severe ID was associated with an increase in TSH stimulation and thyroid size and a reduced risk of hypothyroidism (5). In those children an intervention trial compared the efficacy of iodized salt alone to iodized salt given with VAS and found greater decreases in TSH and thyroid volume in the IS+VAS group (5). In a recent study by Zimmermann et al. which was performed to investigate the effects of supplementation with iodine or VA alone, and in combination, in South African children with concurrent VAD and ID it was found that VAS alone in iodine-deficient children with mild VAD reduced circulating TSH, serum thyroglobulin, and thyroid size without significantly affecting thyroid hormone concentrations (*20*). It was also shown that mild ID did not impair the SR or retinol binding protein (RBP) response to VAS in children with concurrent ID and VAD. Conversely, the

data also indicated that mild VAD did not reduce the efficacy of IS to correct thyroid dysfunction in children with concurrent ID and VAD (*20*). These latter findings differed somewhat from those of several animal studies in which severe VAD impaired the pituitary-thyroid axis, even when the iodine supply was adequate. The adverse effects in these studies included reduced thyroidal iodine uptake (*10*), impaired synthesis of thyroglobulin and coupling of iodotyrosine residues to form thyroid hormone, and reduced hepatic conversion of T4 to triiodothyronine (T3) (*11*). In another study by Biebinger et al. in rats with only mild to moderate VAD and ID, provision of an iodine-sufficient diet entirely reversed the abnormalities of the pituitary-thyroid axis produced by VA and iodine depletion, regardless of VA status (*19*). In iodine-sufficient animals it was shown that pharmacologic doses of VA did affect the pituitary-thyroid axis and decreased thyroid size, pituitary TSH content, and circulating total T3 and total T4 (TT4) (*7*, *36*). A similar effect was reported in lymphoma patients who developed hypothyroidism after treatment with a synthetic retinoid that specifically binds to the RXR (RXR-selective retinoid LG1069) (*35*). VAD can affect thyroid function in some other ways too. Ingenbleek (*11*) reported that a combination of VAD and ID in rats impaired thyroid hormone synthesis by reducing iodine incorporation into thyroglobulin; these adverse effects were reversed by VA treatment. On the other hand, Morley et al. (*36*) found that high-dose VAS in iodine sufficient rats altered peripheral thyroid hormone metabolism and increased hepatic conversion of T4 to T3.

As indicated by the present study there wasn't any significant difference between SR levels in goitrous and nongoitrous children. There wasn't any significant difference between the prevalence of subjects with low VA status in the two groups either. Our findings are different from a study in Moroccan children in which increasing VAD severity was a predictor of greater thyroid volume and higher concentrations of TSH, thyroglobulin, and TT4 (5). Our study also had different results from another study in Ethiopian children, in which there was a strong negative correlation between increasing severity of goiter and SR (*37*). These differences mostly originate from the different degree of VAD in our study in comparison with those studies; whilst in our study there was no child with VAD, previous studies contained more VA-deficient subjects.

Although the interaction between VA deficiency and Fe metabolism is complex (*38*), a positive effect of VA supplementation on Fe status was shown (*39*). In the present study children with low VA status had significantly lower serum iron than subjects with sufficient VA status and SR was correlated with serum iron levels.

The main limitation of our study was that we categorized participants into goitrous and nongoitrous groups by inspection and palpation. It has been stated that in areas of mild to moderate ID, the sensitivity and specifity of palpation are poor (*40*). Classification of subjects into different goiter groups would be more accurate if we used thyroid ultrasonography instead of inspection and palpation. We also didn't investigate the dietary intake of VA in children.

In this study we showed that goiter is still endemic in Semirom. ID alone can not explain the still high prevalence of goiter in this region. VAD or low VA status is not among the contributors of endemic goiter in schoolchildren of Semirom. Therefore we suggest investigating the possible role of other contributors such as protein-energy malnutrition, zinc deficiency, and thiocyanate overload in this region.

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