Original Article

A community-based case–control study to investigate the role of iron deficiency in the persistence of goiter

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ABSTRACT

Objectives: To find out the magnitude of iron deficiency anemia in the age group of 6–12 years and investigate the role of iron deficiency as a possible contributor to endemic goiter in school children in Ambala. **Materials and Methods:** The present study was conducted as a subset of a cross-sectional study among 2700 children from 6 to 12 years of age to find out the prevalence of goiter. All the subjects who were found to be suffering from goiter in the cross-sectional study were enrolled in the case–control study as cases and were compared with age- and sex-matched controls (children without goiter) from the same cohort. The study was conducted from February 2011 to January 2012. **Results:** Out of total, goiter was observed in 12.6% of the subjects. Urinary iodine excretion was found to be <100 μg/L in 57 (10.5%) children. Mean hemoglobin (Hb) level of the study population was 11.9 g/dL. It was noted that 71% of the goitrous children had anemia (Hb <12 g/dL) as compared to 63.7% of the control group. Serum ferritin (SF) was <15 ng/mL in 70% of the children. The mean ± standard deviation of SF in the goitrous and nongoitrous children was 19.65 ± 32.51 μg/L and 27.55 ± 21.07 μg/L, respectively (*P* = 0.012). **Conclusion:** The findings in the study suggest that iron deficiency anemia in children is contributing toward the persistence of goiter in the postiodization phase.

Key words: Goiter, hemoglobin, iodine, iron deficiency, serum ferritin

INTRODUCTION

Iodine deficiency disorders (IDDs) are a worldwide major public health problem. National Iodine Deficiency Disorders Control Programme initially known as National Goitre Control programme was launched way back in 1962 to curb the situation. Even after 53 years of the implementation of the programme, a recent nationwide survey revealed that out of 324 districts surveyed, 263 districts were IDD endemic.[1]

Persistence of goiter in spite of universal iodization of salt for the past so many years indicates that there is a possible role of some other factor which is limiting the effects of iodine supplementation. Several micronutrient deficiencies have been incriminated as the cause of goiter other than iodine; notable among these are iron and selenium deficiencies.[2] The first two steps of thyroid hormone synthesis are catalyzed by thyroperoxidases and are dependent on iron. Animal and human studies suggest that iron deficiency impairs thyroid metabolism. Iron

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deficiency anemia (IDA) decreases plasma thyroxine (T4) and triiodothyronine (T3) concentrations, reduces peripheral conversion of T4–T3, and may increase the concentrations of thyrotropin.[3-6] In addition, iron deficiency may alter central nervous system control of thyroid metabolism and modify nuclear triiodothyronine binding.[3,4] Deficiencies of iron and iodine are major overlapping public health problems in the developing world, where many children are at a high risk of both goiter and IDA.[2]

General malnutrition, water-borne goitrogens, and a variety of goitrogenic food can also aggravate goiter. Iron deficiency was associated with a high prevalence of goiter in Iranian school children and similar results were shown in sub-Saharan Africa.[7] It has been observed that supplementation of iron may improve the efficacy of oral iodized oil in goitrous children with iron-deficiency anemia.[8]

Studies are highly suggestive of a causal relationship, but there is no conclusive evidence that iron deficiency with or without anemia is directly associated with thyroid function. This study is an attempt toward providing health administrators and policy makers with reliable population-based information on the magnitude of the iron deficiency anemia in the age group of 6–12 years and investigate the role of iron deficiency as a possible contributor to endemic goiter in school children in Ambala after 49 years of the initiation of salt-iodization program.

MATERIALS AND METHODS

The present community-based case–control study was conducted as a subset of a cross-sectional study carried out to study the prevalence of goiter in Ambala from February 2011 to January 2012. In the cross-sectional study, a multistage cluster sampling method was adopted for selecting 2700 children in the age group of 6–12 years in the district under study. It included school children and out of school children from the community in the ratio of 3:1. Those children were screened by trained physicians for goiter by standard palpation method and were graded according to the criteria recommended by the joint WHO/UNICEF/ICCIDD.[9] The grading is described as Grade 0 when there is no palpable or visible goiter; Grade 1, goiter that is palpable but not visible when the neck is in the normal position; and Grade 2, goiter is visible when the neck is in the normal position. We excluded children with a history of exposure to radioactive iodine, thyroid surgery, or significant underlying disease, such as cardiopulmonary, liver, or renal problems based on the available medical records and interviewing parents and teachers.

To investigate the role of iron in the persistence of goiter, a case–control study was conducted simultaneously in which 270 children who were detected with goiter in the cross-sectional study were enrolled as cases and similar number of children of same age and sex group but without goiter were selected as controls from the same cohort for comparison. All the selected cases and controls were subjected to hemoglobin (Hb), serum ferritin (SF), and urinary iodine estimation. Three levels of consent were taken: First one from the school authority of Ambala, second from principals of each school surveyed, and finally from the parents of the students whose blood and urine samples were taken. Only those children whose parents provided informed written consents were included in the study.

The following investigations were done using standard methods in 540 (270 cases and 270 controls) children enrolled for the study.

Urinary iodine concentration
Urinary iodine concentration is a well-accepted, easily obtainable, and an effective indicator of a population's iodine nutrition, and the WHO has recommended its use as an impact indicator, namely a marker used to monitor the effects of an intervention on the iodine status of a population. It is considered a sensitive marker of current iodine intake and can reflect recent changes in iodine status. Because urinary iodine values tend not to be normally distributed, the Median Urinary Iodine Concentration (MUIC) is the preferred measure of central tendency and it is most commonly used to describe the distribution of data in the population.[9] The cutoff values to describe a population iodine deficient or sufficient is given in Table 1. For estimation, urine samples were collected in labeled plastic bottles (50 ml capacity with screw cap and thymol crystal as preservative) and taken to the IDD cell, Karnal, for quantitative estimation of iodine in urine. Urine iodine concentration (UIC) was measured by the digestion method based on a modification of Sandell–Kolthoff reaction.

Serum ferritin and hemoglobin concentration
To assess iron deficiency anemia among cases and controls, WHO/UNICEF guidelines were used. It states that if the prevalence of iron deficiency in a population must be described with a single number, SF should be used and complemented

Table 1: Prevalence of goiter among study population			
Grades of goiter	Male *n* (%)	Female *n* (%)	Total *n* (%)
Grade 0	1204 (51)	1156 (49)	2360 (87.4)
Grade 1	114 (4.2)	157 (5.8)	271 (10)
Grade 2	32 (1.2)	37 (1.4)	69 (2.6)
Total	1350	1350	2700

with Hb in all program evaluations.[10] For the estimation of SF and Hb, under aseptic condition, 2 ml of blood was collected from ante-cubital vein in EDTA vacutainers. EDTA samples were processed for Hb estimation within 6 h of collection of the samples. Hb was determined by colorimetric method. SF was also estimated using immunoradiometric assay. Anemia was classified based on the WHO recommended cutoff value of <12.0 g/dL for Hb. Hb concentration <7.0 g/dL was considered severe anemia, 7.0–10.0 g/dL as moderate anemia, and Hb >10.0 g/dL to <12.0 g/dL as mild anemia. A cutoff value of 15 µg/L for SF was chosen based on the recommendations of the WHO for the diagnosis of iron deficiency.[10]

Statistical analysis

Data processing and analysis were done by using SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. (Chicago: SPSS Inc.). Number and percentages were calculated for categorical data. Fisher's exact test and Chi-square test were applied as a test of significance. Mean and standard deviation of urinary iodine, SF, and Hb were calculated and compared by Student's *t*-test. Correlation coefficient of SF and urinary iodine concentration was also calculated.

The study protocol was approved by the Ethical Committee of the institute. Directorate General Health Services, Haryana, was also contacted to provide logistic support and facilitate urinary iodine investigation at state IDD cell laboratory at Karnal, Haryana.

RESULTS

In the cross-sectional study, 2700 school children were enrolled whose age ranged from 6 to 12 years. The mean age of the children was 8.88 ± 1.83 years. Goiter was observed in 340 (12.6%) subjects. Among them, 146 (5.4%) were boys and 194 (7.2%) were girls. In the case–control study, all the cases of goiter could not be enrolled as parents of only 270 of them gave consent for taking blood samples of their children. The basic characteristics of the study population are given in Table 2.

Status of iodine nutrition

On urinary iodine estimation of 540 children (270 cases and 270 controls), the MUIC of the cases and controls was found to be 136.64 µg/L and 199.50 µg/L, respectively. It was found that urinary iodine concentration was <100 µg/L in 57 (10.5%) children only. Thus, based on the epidemiological criterion, the study population had no iodine deficiency [Table 3].

Status of iron deficiency anemia

Of the 540 school children enrolled in the study, 380 (70%) had iron deficiency (SF <15 ng/mL).

The SF (mean ± standard deviation [SD]) level was 18.3 ± 12.3 ng/mL without any significant differences between the boys and girls (19.5 ± 12.4 vs. 17.8 ± 23.4; *P* = 0.205). Mean Hb level of the study population was 11.2 g/dL.

The mean ± SD of SF in the goitrous and non-goitrous children was 19.65 ± 32.51 µg/L and 27.55 ± 21.07 µg/L, respectively (*P* = 0.012). The SF level in the goitrous and nongoitrous girls was 15.53 ± 20.23 µg/L and 18.76 ± 35.20 µg/L, respectively (*P* = 0.57). SF in the goitrous and nongoitrous boys was 19.5 ± 12.4 µg/L and 34.93 ± 23.99 µg/L, respectively (*P* = 0.015). It was noted that 192 (71%) goitrous children had anemia (Hb <12 g/dL) as compared to 172 (63.7%) goitrous children of the control group (odds ratio - 2.010, 95% confidence interval 1.32–3.50, *P* = 0.007) [Table 4].

The SF level and the Hb concentration varied significantly between cases and controls [Table 4].

Table 2: Distribution of subjects according to urinary iodine concentration (*n*=540)

Grades of goiter	Urinary iodine (µg/L)		
	>100	50-100	<50
Grade 0	268	2	0
Grade 1	163	41	0
Grade 2	52	12	2
Total (%)	483 (86.4)	55 (13)	2 (0.3)

Table 3: Baseline characteristics of cases and controls

Characteristics	Goitrous group (*n*=270)	Nongoitrous group (*n*=270)	*P*
Age (mean) in years	8.4±1.4	8.1±1.4	0.84
Sex (male:female)	1:1.3	1:1.2	0.64
Anemia (hemoglobin <11 g/dL) (%)	192 (71)	172 (63.7)	0.01
Serum ferritin (mean) (µg/L)	19.65±32.51	27.55±21.07	0.012
UIC (mean) (µg/L)	186.64±17.21	199.64±16.28	0.062

UIC: Urinary iodine concentration

Table 4: Serum ferritin and hemoglobin level among cases and controls

	Cases (%)	Controls (%)	Total (%)
SF (µg/L)			
SF <15	217 (80.3)	163 (60.3)	380 (70)
15.1 < SF <30	37 (13.7.7)	83 (30.7)	120 (22)
30.1 < SF <45	9 (3.3)	18 (6.6)	27 (5)
SF >45	7 (2.5)	6 (2.2)	13 (2.4)
Total	270	270	540
Hemoglobin (g/dL)			
>11	78 (28.8)	98 (36.2)	176 (32.5)
11-7	172 (63.7)	168 (62)	340 (62.9)
<7	20 (7.4)	4 (1.5)	24 (4)
Total	270	270	540

SF: Serum ferritin

Correlation between urine iodine concentration and serum ferritin

The subjects were then stratified according to their SF level using a cutoff point of 15 μg/L. Children in the first distributional quartile of SF concentration had a lower UIC than children in the fourth quartile (112.30 ± 84.60 vs. 224.54 ± 102 μg/L). SF correlated with UIC (*r* = 0.23, *P* = 0.001) in cases as well as controls [Table 5].

DISCUSSION

In the present study, the urinary iodine excretion level of 100 μg/L and above was found in almost 86.4% of the samples. The median urinary iodine level was 146 μg/L in the current study. These findings indicated that only 57 of the children in the study had a biochemical deficiency of iodine. It also indirectly indicated that there were continued, although inadequate efforts, to ensure a supply of iodized salt to the population. Authors of other studies in India indicated different median urinary iodine levels, which pointed to either a deficiency or no deficiency for certain populations in their areas.[11-14] A study from Nepal reported a 93.5 μg/L median urinary iodine level.[15] An international study from Australia reported a median urinary iodine level of 82 μg/L.[16] Another international study from Lesotho reported a median urinary iodine level of 26.3 μg/L, indicating mild-to-moderate iodine deficiency in other countries.[17]

Although deficiencies of iron and iodine are major overlapping public health concerns in developing countries, previous studies of the relation between iron deficiency and goiter are limited. Iron deficiency state is an important cause for the persistence of goiter in the iodine replete population.[7,8] Compared with healthy control subjects, iron-deficient adults have lower circulating T4 and T3 concentrations and higher thyrotropin concentrations.[4,6,7] Although the mechanism for these effects is unclear, the initial steps of thyroid hormone synthesis—iodide incorporation into tyrosine residues of thyroglobulin and covalent bridging of the residues—are catalyzed by heme-containing thyroperoxidases. Other iron-containing enzymes (e.g., cytochrome c oxidase, myeloperoxidase, and succinate dehydrogenase) are

sensitive to depletion of iron.[18] Theoretically, severe iron deficiency could lower thyroperoxidase activity and interfere with thyroid hormone synthesis.

Hess *et al.* have shown that supplementing iron in iron-deficient children with goiter decreases its size.[9] In the another study in Chandigarh, anemia and iron deficiency as assessed by SF levels were significantly more prevalent in goitrous children than in controls in both the age groups (6–12 and 13–16 years), and SF levels negatively correlated with the presence of goiter.[19]

It was observed in the present study that 71% of the goitrous children had anemia (Hb <11 g/dL) as compared to 63.77% of the nongoitrous group and about 80% of cases had SF level <15 compared to 60.3% of the controls. Contrary to findings of the present study, a survey of Ethiopian children showed no correlation between iron status and goiter rate or thyroid hormone concentrations.[20] A study conducted in Philippines also reported no statistically significant differences in the prevalence of goiter in anemic and nonanemic children and adults.[21] In another study among Ethiopian children who were severely Vitamin A deficient, low T3 concentrations were associated with low serum iron and low transferrin saturation.[22] Similar to our study, iron deficiency was associated with a high prevalence of goiter in Iranian school children.[23]

The mean SF in our study was 18.3 ± 12.3 ng/mL; 70% of the school children had SF <15 ng/mL which was much higher than that reported from other developing countries (25–35%).[24,25]

Although this study was conducted in a community-based setting, utmost care was taken in selecting age- and sex-matched controls. All the investigations were carried out using standard methods and data were analyzed with appropriate statistical tools, however there were certain limitations too. One evident limitation of our study was that participants were grouped into goitrous and nongoitrous groups by inspection and palpation. It has been stated that, in areas of mild-to-moderate IDD, the sensitivity and specificity of palpation are poor.[9] Classification of children into different goiter groups would have been more accurate, had we used thyroid ultrasonography instead of inspection and palpation. Moreover, no hormonal estimation was conducted among children with Grade 2 goiter due to logistic constraints. Even diseases such as autoimmune thyroiditis and presence of goitrogens in the diet could also have accounted for some of the cases of goiter in the present study. Iron deficiency in the anemic subjects was confirmed by using SF and Hb. Because of technical and financial considerations in the field, we were unable

Table 5: Urinary iodine concentration based on quartile of serum ferritin in goitrous and nongoitrous children

SF (μg/L)	UIC in cases (goitrous) (μg/L)	UIC in controls (nongoitrous) (μg/L)
SF ≤15	112.30±84.60	132.3±82
15.1 < SF ≤30	167.07±137.80	182±147.80
30.1 < SF ≤45	203.09±112.07	213.09±102.07
SF >45.0	224.54±102.68	228.54±101.68

UIC: Urinary iodine concentration, SF: Serum ferritin

to include other indicators of iron status such as serum iron, total iron-binding capacity, serum-soluble transferrin receptor, or transferrin concentration besides SF level. SF may be the most useful laboratory measure of Fe status; a low value is diagnostic of IDA in a patient with anemia. It is widely available, well-standardized, and has repeatedly been demonstrated to be superior to other measurements for identifying IDA. There are some situation where SF level is increased independent of Fe status. It can be high in acute and chronic inflammations. It is also unreliable in the setting of malignancy, hyperthyroidism, liver disease, and heavy alcohol intake. This can be adjusted with serum C-reactive protein (CRP) levels, a marker of infection. Although we did not measure CRP, these aspects were taken care of in the present study by including school-going apparently healthy children and excluding children with malignancy, hyperthyroidism, and liver diseases.

CONCLUSION

Findings of the present study demonstrate that though the prevalence of goiter was high (12.6%) among school children in Ambala district and constitutes a public health problem in this region, MUIC indicates that iodine nutrition is adequate. Since iron deficiency was significantly higher in goitrous cases than their controls, the findings in the study also advocate that iron deficiency contributes toward limiting the effect of iodine supplementation program for the control of IDDs as available iodine remains underutilized in presence of iron deficiency anemia. Further studies are needed to understand the role of iron deficiency on the pathogenesis of IDDs because it may have even greater effect on IDDs than any other factors due to the high prevalence of IDA in vulnerable groups such as children and pregnant women. Thus, iron may be only one of the many nutritional and environmental factors that influence the pathogenesis of IDDs in iodine-deficient areas.

Conflicts of interest
There are no conflicts of interest.

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