

Introduction of Iodized Salt to Severely Iodine-Deficient Children Does Not Provoke Thyroid Autoimmunity: A One-Year Prospective Trial in Northern Morocco

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To determine if introduction of iodized salt induces thyroid autoimmunity in goitrous children, we conducted a prospective trial in iodine-deficient Moroccan schoolchildren ($n = 323$). Local salt was iodized at 25 μg iodine per gram of salt and distributed to households. Before introduction of iodized salt and at 10, 20, 40, and 52 weeks, we measured antithyroid peroxidase antibodies (TPO-Ab), antithyroglobulin antibodies (Tg-Ab), urinary iodine (UI), and thyroid hormones, and examined the thyroid using ultrasound. At baseline, median UI was 17 $\mu\text{g}/\text{L}$ and the prevalence of goiter and hypothyroidism was 72% and 18%, respectively. Provision of iodized salt maintained median UI at 150–200 $\mu\text{g}/\text{L}$ for the year ($p < 0.0001$). There was a significant increase in mean total thyroxine (T_4) and a significant reduction in the prevalence of hypothyroidism ($p < 0.001$). There was a transient increase in the prevalence of detectable antibodies after introduction of iodized salt ($p < 0.0001$) with levels returning to baseline at 1 year. Only $\cong 1\%$ of children had elevated TPO-Ab and none had elevated Tg-Ab over the course of the study, and no child with elevated TPO-Ab had abnormal thyrotropin (TSH) or T_4 concentrations. None developed clinical or ultrasonographic evidence of thyroid autoimmune disease and/or iodine-induced hypothyroidism or hyperthyroidism. Rapid introduction of iodized salt does not provoke significant thyroid autoimmunity in severely iodine-deficient children followed for 1 year.

Introduction

THE IODINE-DEFICIENCY DISORDERS (IDD) are the single most important preventable cause of brain damage worldwide (1). Universal salt iodization (USI) is the preferred IDD control strategy and has clear benefits on learning ability and work performance (1). However, there are risks associated with introduction of iodine into areas of longstanding IDD (2). The most common adverse effect is iodine-induced hyperthyroidism, usually occurring in older adults with nodular goiters (3). Another adverse effect may be induction and/or aggravation of thyroid autoimmunity (4). Epidemiologic studies have suggested that antithyroid antibodies are more common in adults in areas of adequate iodine intake than in areas of iodine deficiency (5,6) but not all studies agree (7). A large Indian study found no correlation between urinary iodine (UI) concentration and either antithyroid antibodies or autoimmune thyroiditis in adolescence (8).

Several intervention studies have suggested increasing iodine intake may enhance thyroid autoimmunity (9–11); others have not (12–15). In the intervention studies that have found a link between iodine and thyroid autoimmunity, the

iodine was given as iodized oil or potassium iodide to adults (9–11). Two previous trials of iodized oil in children reported no induction of thyroid autoimmunity (13,14). We are aware of no published prospective studies examining whether introduction of iodized salt to an area of endemic goiter induces thyroid autoimmunity in children, a major target group of IDD control. Therefore, we conducted a prospective trial of iodized salt in an area of endemic goiter in the mountains of northern Morocco. In severely iodine-deficient children, before introduction of iodized salt and for 1 year afterward, we measured antithyroid peroxidase antibodies (TPO-Ab), antithyroglobulin antibodies (Tg-Ab), UI and thyroid hormones, and examined the thyroid using ultrasound.

Subjects and Methods

The study was done in rural villages in the Rif Mountains of northern Morocco. This is a region of longstanding severe IDD, where the goiter rate among schoolchildren is 53%–72% (16,17). Salt is likely to be an effective vehicle for fortification in this region, because it is widely and regularly consumed at a level of 5–12 g/d (18). The subjects were 6–15-

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year-old children from two neighboring primary schools. All children in the schools were invited to participate in the study, all accepted and were enrolled ($n = 323$). Informed oral consent was obtained from the chief medical officer of the province, the school director, and the parents of the children. The University Children's Hospital in Zürich and the Ministry of Health in Morocco gave ethical approval for the study. At baseline, weight and height were measured and a casual spot urine sample was collected for measurement of UI. The thyroid was examined and its volume measured using a portable Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a high-resolution 7.5-MHz linear transducer (1). Whole blood was spotted onto filter paper for measurement of total thyroxine (T_4), thyrotropin (TSH), antithyroid peroxidase antibodies (TPO-Ab), and antithyroglobulin antibodies (Tg-Ab). Updated World Health Organization/International Council for the Control of Iodine Deficiency Disorders (WHO/ICCIDD) references for thyroid volume by gender and body surface area were used to classify goiter (22).

The salt for this region is supplied by a small local cooperative and has a native iodine content of less than 2 ppm (17). For this study, reagent-grade potassium iodide (Sigma & Aldrich, Buchs, Switzerland) was dry-mixed into the local salt using a rotating drum mixer (Engelsmann, Ludwigshafen, Germany), at a level of 25 μg iodine per gram of salt. Each household with children enrolled in the study was provided with 2 kg of iodized salt monthly for 1 year. The salt was dispensed directly to the head of the household from a central supply at the local health center. At 10, 20, 40, and 52 weeks, weight and height were remeasured, spot urine samples were collected for UI, and whole blood was spotted onto filter paper for determination of T_4 , TSH, TPO-Ab, and Tg-Ab. The thyroid was examined with ultrasound and the children were questioned regarding any changes in thyroid tenderness and examined for eye signs consistent with thyroid autoimmunity.

Laboratory methods

Urine samples were aliquoted and frozen at -20°C until analysis. UI was measured using a modification of the Sandell-Kolthoff reaction (19). Thyroid volume was calculated using the method of Brunn et al. (20). Dried blood spots on filter paper were analysed for TSH using immunoassay (21) and serum T_4 (DELFI Neonatal T_4 Kit, PerkinElmer Life Sciences, Wallac, Turku, Finland). Normal reference values are whole blood TSH, 0.2–3.7 mU/L; serum T_4 , 65–165 nmol/L. We classified thyroid status as follows: subclinical hypothyroidism: TSH greater than 3.7 mU/L and T_4 65–165 nmol/L; overt hypothyroidism: TSH greater than 3.7 mU/L and T_4 less than 65 nmol/L; compensated hypothyroidism: TSH 0.2–3.7 mU/L and T_4 less than 65 nmol/L. TPO-Ab and Tg-Ab were measured by radioimmunoassay (RIA TgAb, RSR, Cardiff, United Kingdom), adapted in our laboratory for measurement on dried blood spots (for the Tg-Ab assay: between assay coefficient of variation [CV] = 10.1%, within assay CV 2.5%; for the TPO-Ab assay: between assay CV = 6.8%, within assay CV = 4.4% [$n=145$]). We classified antibody status as follows: detectable: TPO-Ab greater than 3 U/mL; Tg-Ab greater than 0.3 U/mL and elevated: TPO-Ab greater than 12 U/mL; Tg-Ab greater than 10 U/mL.

Statistical methods

Data processing and statistics were done using Prism3 (GraphPad, San Diego, CA) and Excel 97 (Microsoft, Seattle, WA). One-way analysis of variance (ANOVA) was done to compare changes in UI, TSH, T_4 , TPO-Ab, Tg-Ab, and thyroid volume, with Tukey test for *post hoc* comparisons. Variables not normally distributed (UI, TSH, TPO-Ab, and Tg-Ab) were logarithmically transformed before analysis. Proportions were compared using the χ^2 -test. Significance was set at $p < 0.05$.

TABLE 1. CHANGES IN TSH, TOTAL T_4 , ANTITHYROID PEROXIDASE ANTIBODIES, ANTITHYROGLOBULIN ANTIBODIES, URINARY IODINE, AND THYROID VOLUME IN SIX- TO FIFTEEN-YEAR-OLD MOROCCAN CHILDREN BEFORE AND AFTER INTRODUCTION OF IODIZED SALT

	Baseline (n = 323)	10 weeks (n = 323)	20 weeks (n = 323)	40 weeks (n = 323)	52 weeks (n = 323)
Whole blood TSH (mU/L) ¹	0.9 (0.4–27.0) ^a	0.9 (0.3–3.2) ^a	1.1 (0.3–8.6) ^b	0.7 (0.3–2.4) ^c	0.7 (0.3–2.3) ^c
Percentage of children > 3.7 mU/L	2	0	1	0	0
Percentage of children < 0.2 mU/L	0	0	0	0	0
Serum T_4 (nmol/L) ²	82.6 \pm 19.2 ^a	96.1 \pm 24.4 ^{b,c}	98.1 \pm 22.5 ^b	93.1 \pm 19.7 ^c	94.3 \pm 19.6 ^c
Percentage of children > 165 nmol/L	0	0	1	0	1
Percentage of children < 65 nmol/L	18 ^a	8 ^b	4 ^b	4 ^b	6 ^b
Antithyroglobulin antibodies ³					
Percentage of children > 0.3 U/mL	10 ^a	19 ^b	21 ^b	20 ^b	10 ^a
Percentage of children > 10.0 U/mL	0	0	0	0	0
Antithyroid peroxidase antibodies ³					
Percentage of children > 3 U/mL	48 ^a	74 ^b	77 ^b	72 ^b	55 ^a
Percentage of children > 12 U/mL	1	1	1	1	1
Urinary iodine concentration ($\mu\text{g/l}$) ¹	17 (0–143) ^a	86 (12–511) ^b	180 (22–529) ^c	187 (14–474) ^c	169 (22–439) ^c
Thyroid volume (mL) ²	9.1 \pm 3.7 ^a	8.9 \pm 3.3 ^a	7.9 \pm 3.0 ^b	6.4 \pm 2.5 ^c	5.8 \pm 2.4 ^d

As median (range)¹ or mean \pm SD²; compared by one-way ANOVA across groups and using Tukey's test for *post hoc* comparisons. As percentage³; compared by χ^2 test. In rows, values without a common letter differ, $p < 0.05$.

TSH, thyrotropin; T_4 , thyroxine; SD, standard deviation; ANOVA, analysis of variance.

Results

Mean age (standard deviation [SD]) of the sample was 10.2 (2.4) years, the age range was 6–15 years, and the male to female ratio was 172:151. At baseline, the subjects were severely iodine deficient: UI was $17 \mu\text{g}/\text{L}$ and 72% of the children were goitrous (1). Fifty-five children (18%) were hypothyroid: 6 were overtly hypothyroid, 4 subclinically hypothyroid, and 45 exhibited compensated hypothyroidism (defined in the "Subjects and Methods" section). Introduction of iodized salt rapidly increased iodine intake to adequate levels after 10 weeks and maintained normal UI for

the remainder of the year ($p < 0.0001$) (Table 1). Median thyroid volume by ultrasound was significantly reduced from baseline at 20, 40, and 52 weeks ($p < 0.001$). The enlarged thyroids exhibited the diffuse, homogeneous hypoechoic pattern of endemic goiter, with less than 2% showing significant nodularity at baseline or during follow-up. Over the course of the study, no child had an echogenic pattern consistent with thyroid autoimmune disease or complained of tenderness during thyroid examination. Also, no child exhibited eye signs consistent with thyroid autoimmune disease. At 20 and 40 weeks, there was a significant increase in mean total T_4 ($p < 0.02$) and a significant reduction in the

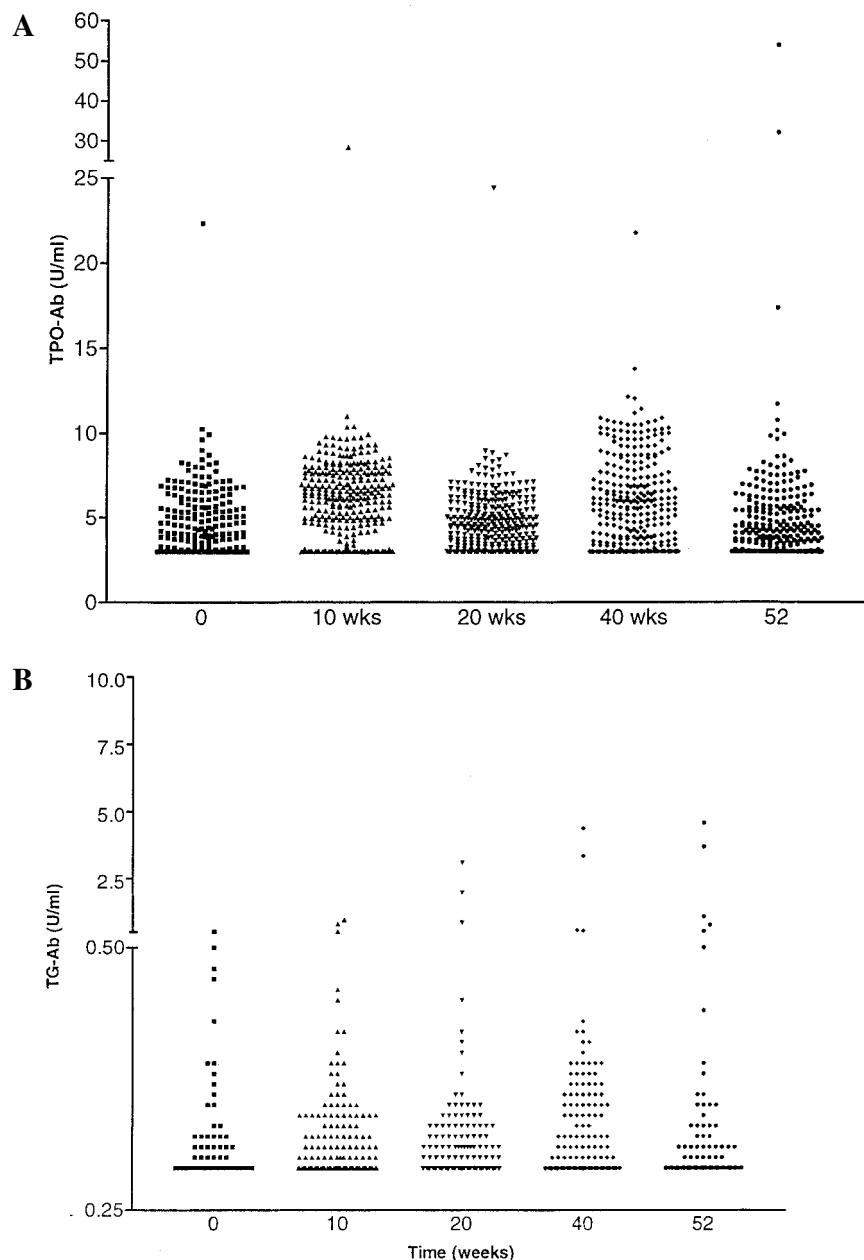


FIG. 1. Antithyroid peroxidase antibodies (TPO-Ab) (**A**) and antithyroglobulin antibodies (Tg-Ab) (**B**) in 323 iodine-deficient Moroccan schoolchildren before (0 weeks) and 10, 20, 40, and 52 weeks after introduction of iodized salt.

number of children with overt and/or compensated hypothyroidism ($p < 0.0001$). No cases of iodine-induced hypothyroidism or hyperthyroidism were evident during follow-up.

Table 1 shows the prevalence of TPO-Ab and Tg-Ab before and after introduction of iodized salt. There was a transient increase in the number of children with detectable TPO-Ab and Tg-Ab in the sample at 10, 20, 40 weeks ($p < 0.0001$), with a return to baseline at 52 weeks. There was no significant increase in the prevalence of elevated TPO-Ab or Tg-Ab in the sample. The prevalence of elevated antibody titers was low: only 1% had elevated TPO-Ab before and after introduction of iodine, and no child had an elevated Tg-Ab during the study period (Fig. 1). Among the children with elevated TPO-Ab, all had normal TSH and T_4 concentrations.

Discussion

Some, but not all, cross-sectional studies have suggested that autoimmune thyroiditis is more common in areas of adequate iodine intake than in areas of iodine deficiency (4,5,23). Laurberg et al. (7) compared elderly subjects in areas of high- and low-iodine intake and found the prevalence of thyroid autoimmunity to be higher in the area of low iodine intake. In an iodine-deficient area of Greece, Tsatsoulis et al. (6) reported an association between higher urinary iodine excretion and thyroid autoimmunity. Marwaha et al. (8) in a sample of 6280 10–18-year-old Indian girls found no correlation between urinary iodine excretion and either antithyroid antibodies or autoimmune thyroiditis by cytology. A study in Sri Lanka found a high prevalence of antithyroglobulin antibodies in 11–16-year-old girls, but no correlation with urinary iodine excretion. Although the authors suggested the recent introduction of iodized salt could be responsible, no preiodine data were available (24).

Data from prospective clinical studies in adults on whether iodine can aggravate or induce thyroid autoimmunity and thyroid dysfunction are equivocal. Boukis et al. (9) gave 1 mL of iodized oil intramuscularly to 58 goitrous adults from a mildly iodine-deficient area of Greece. Thyroid autoantibodies were undetectable in all subjects before treatment. During 6 months of follow-up, 43% of subjects developed positive titers of either TPO-Ab and/or Tg-Ab. Three of the subjects who developed thyroid autoantibodies also developed transient hypothyroidism. In 30 goitrous adults treated with 0.3 mg iodine per day as potassium iodide, 9 (30%) developed elevated thyroid antibody titers during 6 months of follow-up. Induction of thyroid antibodies appeared to be dose-dependent, because only 12% of goitrous subjects receiving 0.15 mg iodine per day became antibody positive (25). In a study by Kahaly et al. (10), 62 goitrous adults were given either 0.2 mg iodine per day as potassium iodide or placebo. Reversible iodine-induced thyroid autoimmunity—elevated microsomal and thyroglobulin antibody titers accompanied by lymphocytic infiltration—was observed in 3 of 31 (9.7%) of subjects. Kahaly et al. (11) gave 0.5 mg of iodine per day as potassium iodide (cumulative dose, 0.09 g of iodine) to 31 adult subjects with endemic goiter. Partly reversible iodine-induced thyroid dysfunction and autoimmunity were observed in 6 of 31 (19%) subjects. In contrast, Tonglet et al. (12) gave oral iodized oil (47 or 118 mg of iodine) to 50 IDD-affected adult

subjects and found no increase in thyroid microsomal or thyroglobulin antibodies.

In our sample, before and after introduction of iodized salt, elevated antithyroid antibodies were rare (~1%) and there was no clinical or ultrasonographic evidence of thyroid autoimmune disease. Previously reported prevalences of elevated thyroid microsomal antibodies (TMA) and Tg-Ab in children vary from 0.6%–3.3% in non-IDD-affected areas to 10.8%–16.5% in goitrous subjects (6,8,26–29). Differences in reported antibody prevalences in children from IDD-affected areas may be the result of interassay variability, differing severity of IDD, and/or differing sample characteristics, including age and gender. In our sample, introduction of iodized salt was associated with a transient increase in the prevalence of detectable TPO-Ab and Tg-Ab at 10, 20, and 40 weeks, with levels returning to baseline at 1 year. As in previous reports, detectable TPO-Ab were 4–5x more common than Tg-Ab (8). In contrast to previous reports of increased TPO-Ab in older females (4), there was no significant gender or age differences in prevalence of detectable TPO-Ab or Tg-Ab in our sample.

Our findings agree with data from two previous studies of iodized oil in children (13,14). Benmiloud et al. (13) administered oral iodized oil in doses ranging from 120–960 mg of iodine to 182 Algerian schoolchildren and found no increase in antithyroid peroxidase antibodies. There was no induction of antithyroid antibodies in 114 iodine-deficient Romanian schoolchildren receiving 200 mg of iodine as oral iodized oil followed for 1 year (14). In the present study, introduction of iodized salt at recommended levels of iodine intake effectively normalized iodine status and improved thyroid function in children without provoking thyroid autoimmunity. These data emphasize the efficacy and safety of the current recommendation for USI to control IDD in schoolchildren.

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