CLINICAL STUDY

Evolution of thyroid autoimmunity during iodine prophylaxis – the Sri Lankan experience

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Abstract

Objective: To study the evolution of thyroid autoimmunity, in relation to the change in goitre prevalence, during 3 years of iodine prophylaxis in Sri Lanka.

Methods: Two groups of Sri Lankan schoolgirls between the ages of 10.8 and 17.5 years were studied in 1998 (401 girls) and 2001 (282 girls). A prospective study was performed in 42 schoolgirls who were thyroid autoantibody (Ab)-positive (+ve) in 1998. Anthropometric measures, urinary iodine excretion (UIE), thyroid volume, free thyroxine, free tri-iodothyronine, TSH, and thyroglobulin (Tg) and thyroid peroxidase (TPO) Ab were evaluated in all 683 girls.

Results: Goitre prevalence was significantly lower in 2001 compared with 1998 related to age (2.9% compared with 20.2%) and body surface area (11.6% compared with 40.8%), although UIE was unchanged. Prevalence of thyroid Ab in 2001 was also lower (23.4% compared with 49.9%); among those with the Ab, 34.8% had TgAb alone and 46.9% had a combination of TgAb+TPOAb, compared with 82.0% TgAb alone in 1998. In 2001, subclinical hypothyroidism was more frequent in Ab+ve (6.3%) than Ab-negative girls (1.0%). A cohort of 42 Ab+ve schoolgirls in 1998 (34 with TgAb alone, eight with TgAb + TPOAb) were evaluated again in 2001. Only 10 of them (23.8%) remained Ab+ve (mostly TPOAb±TgAb) in 2001.

Conclusions: This study demonstrates that: (1) in 2001, goitre prevalence and thyroid autoimmunity rates were significantly lower than in 1998; (2) the pattern of thyroid Ab was different in the two surveys; (3) in 2001 alone, the occurrence of hypothyroidism was correlated with the presence of thyroid autoimmunity. These results indicate an evolution of thyroid autoimmune markers during the course of iodine prophylaxis, which has not been described before.

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Introduction

Universal salt iodination was introduced in 1993 in Sri Lanka, because of a high prevalence of endemic goitre (1). The effects of salt iodination had been examined in a previous study in 1998, in which we reported a high prevalence of thyroglobulin antibodies (TgAb) in schoolgirls from areas with previous low, intermediate or high prevalence of goitre (2). There is convincing epidemiological and clinical evidence to suggest that iodine supplementation in an iodine-deficient population may precipitate the onset of thyroid autoimmunity (2–9). In addition to our own data, there are data from Greece showing an increase in lymphocytic infiltration of the thyroid and an increase in the prevalence of thyroid antibodies (Ab) after iodine supplementation (3, 7). Furthermore, there is also evidence

to suggest that the predominant form of non-toxic goitre, after iodine prophylaxis in previously iodine-deficient areas of Greece, is autoimmune thyroiditis (6). Despite these data, there remains a debate as to the relationship between endemic goitre, iodine prophylaxis and thyroid autoimmunity. The short-term modulatory effects of iodine on thyroid autoimmunity have been demonstrated in both experimental and clinical studies (5, 8, 10, 11), but doubt remains as to the role of thyroid morphological abnormalities (such as the presence of a goitre) in favouring the induction of such autoimmunity (12, 13). There is also debate about the evolution of immune changes in the course of iodine prophylaxis.

In the present study we re-evaluated the thyroid status of Sri Lankan schoolgirls 3 years after the first survey (2). The aim was to investigate whether

the prevalence and the clinical presentation of thyroid autoimmunity in this population had changed during the course of iodine prophylaxis in relation to the reduction in goitre prevalence. We present the results of the 2001 study and highlight important changes from 1998.

Materials and methods

Study procedure

We examined a total of 683 girls between the ages of 10.8 and 17.5 years, from five different regions of Sri Lanka, chosen for the low (two regions), intermediate (one region) and high (two regions) prevalence of goitre as indicated in a previous study (14). Four hundred and one girls were examined in January 1998 and 282 were studied in January 2001 (Table 1). The two groups of schoolgirls were of a similar ethnic background and from regions comparable for iodine status and former prevalence of goitre (14). A cohort of 42 schoolgirls (all positive for thyroid Ab in 1998) were studied sequentially during the 3-year follow-up. The local ethics committee approved the project and the government and the schools involved granted permission for investigations in schoolchildren.

Weight, standing height and the right triceps skinfold thickness were measured as previously described (15). The body surface area (BSA: m²) was calculated using the formula (16):

$$\text{BSA} = \text{weight (kg)}^{0.425} \times \text{height (cm)}^{0.725} \times 71.84 \times 10^{-4}$$

The formula for calculating body fat was (17):

$$\text{body fat (\%)} = 53.09 + (0.14 \times \text{weight}) + (-0.3 \times \text{height}) + (0.88 \times \text{triceps})$$

The lean body mass (LBM) was calculated as the reciprocal of body fat in relation to weight.

Table 1 Clinical and anthropometric profiles of the schoolgirls studied in 1998 (*n* = 401) and 2001 (*n* = 282). Data are expressed as mean ± s.d.

Parameter	1998 Survey (<i>n</i> = 401)	2001 Survey (<i>n</i> = 282)	<i>P</i>
BSA (m ²)	1.26 ± 0.1	1.36 ± 0.1	<0.001
LBM (%)	28.3 ± 4.9	32.4 ± 7.7	<0.001
BF (%)	24.4 ± 4.9	23.8 ± 4.3	0.1
Age (years)	13.7 ± 1.3	15.0 ± 1.2	<0.001
Thyroid volume (ml)	7.7 ± 3.7	5.8 ± 2.2	<0.001

BSA, body surface area; LBM, lean body mass; BF, body fat.

A venous blood sample was collected for the estimation of free thyroxine (fT₄), free-tri-iodothyronine (fT₃), thyrotropin (TSH), TgAb and thyroid peroxidase (TPO) Ab. These blood samples were centrifuged immediately and serum was stored at -20 °C. A random sample of urine (20 ml) was collected for the estimation of urinary iodine excretion (UIE). These samples were also stored at -20 °C.

Ultrasonographic scans of the thyroid gland were undertaken by two experienced operators (PS, HA) using a portable ultrasound machine fitted with a 7.5 MHz transducer (Siemens SL, Erlangen, Germany). In each case, ultrasound was performed with the individual in the supine position with the neck hyper-extended. The volume (ml) of each lobe of the gland was calculated according to the formula:

$$\text{width (cm)} \times \text{length (cm)} \times \text{thickness (cm)} \times 0.479$$

and the lobe volumes were added. The volume of the isthmus was not included. Thyroid glands were classified as normal or enlarged using the corrected World Health Organization/International Council for Iodine Deficiency Disorders (WHO/ICCIDD) reference data [multiplying the current WHO/ICCIDD values (18) by 0.71 as described by Zimmerman *et al.* (19)]. Thyroid volumes greater than the 97th percentile were considered to be increased. The denominators for calculating the prevalence of increased thyroid volume are based on those individuals with either age or BSA within the ranges represented in the respective WHO/ICCIDD data sets. Therefore, only those individuals aged 11–15 years (350 from the 1998 survey, and 176 from the 2001 survey) or those with BSA 0.8–1.7 m² (all 401 from the 1998 survey, and 279 from the 2001 survey) were used in the relevant prevalence calculations.

Biochemical assays

fT₃, fT₄ and TSH were measured using an automated immunoassay analyser, the ACS-180 Plus (Chiron Diagnostic Ltd, Halstead, Essex, UK). fT₄ and fT₃ were measured with competitive labelled antibody assays utilising an acridium ester as label and paramagnetic particles as solid phase, and TSH was measured using a two-site immuno-chemiluminometric assay. Normal values were as follows: fT₃ 3.5–6.8 pmol/l, fT₄ 9.8–23 pmol/l and TSH 0.35–5.2 mU/l. The between-batch precisions of the assays were as follows: fT₄ (mean 13.6 pmol/l) coefficient of variation (CV) = 4.0%, fT₃ (mean 5.27 pmol/l) CV = 4.85%, and TSH (mean 4.89 mU/l) CV = 7.56%. TgAb (normal value <98 kIU/l) and TPOAb (normal value <19.4 kIU/l) were measured by ELISA, as described previously (2). The same procedures and antigens were used in 1998 and 2001. In addition, 108 random samples from the 1998 survey were re-assayed in the same batch as the 2001 samples, for TgAb. The differences in values

between the two assays were within the ranges of variation described previously (20). The intra-assay variation for TgAb was 4.8% (mean 1350 kIU/l) and that for TPOAb was 4.9% (mean 155 kIU/l). The interassay variation for TgAb was 7.2% (mean 1387 kIU/l) and that for TPOAb was 7.6% (mean 149 kIU/l). UIE was measured using a manual method based on a modification of Barker's dry ash technique (21); results are expressed directly as $\mu\text{g/l}$ urine.

Statistical analysis

The data were expressed as mean $\pm$ standard deviation (s.d.). For thyroid volume, a logarithmic transformation was used to normalise the distribution. The Kolmogorov–Smirnov test was applied to check normality of the transformed variable in each age and BSA group. All Kolmogorov–Smirnov tests were non-significant. Means and s.d. of the logarithm of thyroid volume were thus used as parameters to fit a normal distribution to the data of each group. Unpaired data were compared using *t*-test. Frequencies were compared using χ^2 test, with Fisher correction when appropriate. Partial correlation was performed to evaluate the differences in LBM and BSA between the two groups without the effects of the age. The longitudinal study was performed using non-parametric tests. Repeated data and frequencies were compared using Wilcoxon's test and McNemar's test, respectively. Logistic regression analysis was performed to assess the likelihood of thyroid Ab positivity in 2001. A probability (*P*) < 0.05 was considered significant.

Results

The mean age, LBM and BSA of schoolgirls from the 2001 survey were greater than those of girls studied in 1998, but the body fat was comparable (Table 1). To detect whether the differences in LBM and BSA between the two groups were age-dependent, a partial correlation was performed. Eliminating the effects of age, girls from the 2001 study still had a greater LBM (*P* < 0.001) and BSA (*P* = 0.001) than those from 1998. In the 2001 survey, mean thyroid volume was 5.8 ± 2.2 ml (range 1.6–19.6 ml), significantly smaller than in 1998 (7.7 ± 3.7 ml, range 2.9–38.5 ml) (*P* < 0.001) (Table 1). Goitre prevalence was calculated applying the corrected WHO/ICCIDD thyroid volume reference ranges (19) for age (Fig. 1a) and BSA (Fig. 1b). The prevalence of goitre based on age was 20.2% [95% confidence interval (CI) 8.69 to 26.5] and 2.9% (95% CI 1.5 to 5.8) in 1998 and 2001 respectively (*P* < 0.001). The prevalence of goitre based on BSA was 40.8% (95% CI 30.6 to 47.6) and 11.6% (95% CI 2.7 to 35.2) in 1998 and 2001 respectively (*P* < 0.001). In both surveys, the prevalence of goitre for BSA was greater than that

Figure 1 Thyroid volume in relation to the age (a) and body surface area (b) in Sri Lankan schoolgirls studied in 1998 (□) and 2001 (■). Thyroid volumes above the 97th percentile (solid line) of the WHO/ICCIDD reference data for age and BSA were considered to be increased (goitre). The prevalence of goitre for age (a) was calculated in the schoolgirls up to age 15.5 years (dashed line) (350 cases in 1998 and 176 cases in 2001). The prevalence of goitre for BSA (b) was calculated in the schoolgirls up to BSA 1.75 m² (dashed line) (all 401 cases in 1998 and 279 cases in 2001).

for age. Overall, among the 150 schoolgirls (133 in 1998 and 17 in 2001) with increased thyroid volume based on BSA, 81 (69 in 1998 and 12 in 2001) had a normal age-related thyroid volume (*P* < 0.001). In 1998, the prevalence of goitre was different in relation to the geographical area (Fig. 2).

No significant difference in UIE was found between the two surveys (149.5 ± 78.4 $\mu\text{g/l}$ and 158.9 ± 69.5 $\mu\text{g/l}$ in the 1998 and 2001 surveys respectively; *P* = 0.34). In 1998, the distribution analysis showed a major asymmetry (1.3 compared with 0.6) and larger range (14–540 compared with 48–300) of UIE values compared with those seen in 2001.

Figure 2 Prevalence of goitre based on age (shaded bars) and BSA (open bars) in schoolgirls studied in 1998 and 2001, from different regions of Sri Lanka. Regions of low, intermediate and high prevalence of goitre are in relation to the rates of goitre reported before the introduction of iodine supplementation (14).

Thyroid autoimmunity and function

In the 2001 survey, 66 of 282 schoolgirls (23.4%) were found to be positive for thyroid Ab (Ab+ve), a significantly lower percentage than that seen in 1998 (49.9%) (χ^2 48.8; $P < 0.001$). Furthermore, the pattern of thyroid Ab was significantly different between the two surveys (χ^2 96.3; $P < 0.001$): the prevalence of isolated TgAb positivity was lower in 2001 than in 1998, whereas the prevalence of isolated TPOAb positivity was higher in 2001 (Fig. 3). In 1998, 82.0% of the Ab+ve girls (164/200) were positive for TgAb alone, whereas in 2001 most of the Ab+ve girls showed positivity for TPOAb, either alone (12/66; 18.2%) or in combination with TgAb (31/66; 46.9%).

In 1998, the different geographic areas showed a significant difference in the incidence of thyroid autoimmunity, with a greater percentage being seen in regions previously reported to have a high prevalence of goitre (76.9%) in contrast to the regions of intermediate (47.7%) or low (40.4%) prevalence of goitre (χ^2 18.9; $P < 0.001$). However, regional variation in the prevalence of thyroid autoimmunity was not seen in 2001 (18.4% compared with 27.9% for the regions of high and low prevalence of goitre respectively; χ^2 3.5; $P = 0.07$).

Overt thyroid dysfunction was not seen in either survey. Subclinical hypothyroidism (high TSH values

Figure 3 Percentage positivity for thyroid autoantibodies in schoolgirls from the 1998 and 2001 surveys. Total Ab prevalence was significantly greater in 1998 than in 2001. The percentage of positivity for TgAb alone (open bars) was significantly lower in 2001 than in 1998 ($P < 0.001$). The positivity for TPOAb alone (black bars) was significantly greater in 2001 than in 1998 ($P = 0.001$). No significant difference was found in TgAb + TPOAb positivity (shaded bars) between the two surveys ($P = 0.29$).

with fT_3 and fT_4 in the normal range) was found in five girls in 1998 (1.2%) and in six in 2001 (2.1%) (χ^2 0.8; $P = 0.4$). Subclinical thyrotoxicosis (low TSH values with fT_3 and fT_4 in the normal range) was found in 12 girls in the 1998 survey (3.0%), compared with only two in 2001 (0.7%) (χ^2 4.3; $P = 0.05$). In 1998, comparable rates of subclinical thyroid dysfunction were found between the Ab+ve and Ab-negative (Ab-ve) girls (1.0% and 1.5% for hypothyroidism; 2.1% and 4.1% for thyrotoxicosis), with lower UIE in the hypothyroid girls ($79.4 \pm 34.3 \mu\text{g/l}$, range 49–129 $\mu\text{g/l}$) and greater UIE in hyperthyroid girls ($194.3 \pm 93.4 \mu\text{g/l}$, range 56–322 $\mu\text{g/l}$) than in euthyroid girls ($147.1 \pm 75.1 \mu\text{g/l}$, range 14–540 $\mu\text{g/l}$). In 2001, however, the prevalence of hypothyroidism was significantly greater in the Ab+ve (6.3%) girls than in the Ab-ve girls (1.0%) (χ^2 6.5, $P = 0.03$), without a significant difference between UIE and euthyroid individuals ($153.5 \pm 70.6 \mu\text{g/l}$ compared with $159.5 \pm 69.8 \mu\text{g/l}$, NS). The two individuals with thyrotoxicosis in 2001 were negative for thyroid autoantibodies.

Forty-two Ab+ve schoolgirls (34 with TgAb alone and eight with TgAb + TPOAb) in 1998 were re-examined in 2001. In 1998, TgAb titres were slightly increased (98–196 kIU/ml) in 54.8% (23/42), moderately increased (196–392 kIU/ml) in 28.6% (12/42) and highly increased (>392 kIU/ml) in 16.7% (7/42). TPOAb positivity was found in all seven girls with highly increased TgAb titres, but in only one of 12 with moderately increased TgAb titres (χ^2 36.0; $P < 0.001$). At follow-up, TgAb was negative in 35 previously TgAb+ve girls (Fig. 4a). Among the eight TPOAb+ve schoolgirls in 1998, four became TPOAb-ve and four remained TPOAb+ve in 2001. A further five became TPOAb+ve during the follow-up (Fig. 4b). In 2001, 10 girls (23.8%) were still Ab+ve and 32 were found to be negative ($P < 0.001$ compared with 1998). The presence of TPOAb in association with TgAb in 1998 predicted the persistence of thyroid autoimmunity in the following years (odds ratio 3.1, 95% CI 1.3 to 7.3; $P = 0.01$). In fact, 62.5% of the schoolgirls with TgAb + TPOAb and only 14.7% of those with TgAb alone in 1998 remained Ab+ve in 2001 (χ^2 8.1; $P = 0.01$). In 2001, only one Ab+ve girl had TgAb alone, whereas nine were TPOAb+ve either alone or in combination with TgAb ($P < 0.001$ compared with 1998). All 42 schoolgirls were euthyroid both in 1998 and in 2001. There was no significant difference in TSH values between the two studies.

Discussion

We have demonstrated a significant decrease in the prevalence of thyroid Ab and goitre in Sri Lankan schoolgirls examined 1998 and 2001, accompanied by a modulation in the pattern of thyroid Ab.

The immune modulatory effects of iodine are well documented (10, 11), and several possible mechanisms may explain these effects. Thyroglobulin is an iodinated compound and is an integral part of the hormone-synthesising mechanisms of the thyroid follicle. Iodinated thyroglobulin has been shown to elicit a greater immune response compared with less iodinated thyroglobulin molecules in *in vitro* experiments (22–24). Recently, it has been demonstrated that the enhanced iodination of thyroglobulin induces the expression of new cryptic epitopes on the molecule that could be responsible for triggering the autoimmune process (25). However, there are no experimental data describing the long-term immune-modulatory effects of iodine, as occurs in populations exposed to continuing iodine prophylaxis.

The prevalence of thyroid autoantibodies was lower in 2001 than in 1998. This result may have been affected by the cross-sectional design of the present study and the fact that we studied two different groups of schoolgirls. However, the two populations were of similar ethnicity (Sinhala), and variability in genetic susceptibility to autoimmune thyroid disease would be negligible. In addition, the schoolgirls from both 1998 and 2001 were from regions of Sri Lanka with comparable iodine status. It is also unlikely that the age difference in the 1998 and 2001 groups affected the prevalence of thyroid Ab, as the difference in age was too small to be clinically relevant. Furthermore, this trend towards a lower prevalence of thyroid Ab was confirmed in the cohort of 42 girls followed sequentially. The greater prevalence of goitre in the 1998 group may have favoured the occurrence of thyroid autoimmunity, as there is evidence to suggest that the mechanisms involved in goitre formation do predispose to thyroid autoimmunity (12). It should be noted that the regions with greater prevalence of goitre in 1998 showed the greatest rate of thyroid autoimmunity. The effect of other environmental factors, such as selenium deficiency, also may have affected the differences in thyroid autoimmunity between the two surveys (26).

Regardless of the above considerations, the differences between 1998 and 2001 could also have reflected a partial reversibility of thyroid autoimmunity during continued exposure to iodine [and analogous to iodine-induced thyrotoxicosis after iodine supplementation (27)]. This is suggested by the longitudinal observation that most of the individuals positive for TgAb alone in 1998 were Ab-ve in 2001. Whether this shift was an expression of the natural history of thyroid disease or the enduring effect of iodine prophylaxis is difficult to assess. Recently, it has been demonstrated that TgAb disappear spontaneously during 3-year follow-up in young individuals with thyroid autoimmunity (28). In our schoolgirls, such a trend may have been favoured by the time-dependent effects of iodine prophylaxis. In fact, the sudden increase in iodine

Figure 4 (a) Individual TgAb values in 42 Sri Lankan schoolgirls who were Ab+ve in 1998. Thirty-five schoolgirls were negative in 2001, whereas seven remained Ab+ve. (b) Individual TPOAb concentrations in 13 Sri Lankan schoolgirls: four were TPOAb+ve in 1998 and TPOAb-ve in 2001, and four were positive at both times; five were TPOAb-ve in 1998 but became TPOAb+ve in 2001. Another 29 schoolgirls (not shown in the graph) showed negative values both in 1998 and in 2001. The dashed lines represent the upper limit of the normal range for TgAb in (a) and TPOAb in (b).

intake that occurs when iodine supplementation is first introduced may have induced the greater prevalence of TgAb in 1998 in comparison with 2001 (29, 30). It is noteworthy that the reversibility of thyroid autoimmunity occurred mainly in those individuals who showed low titres of TgAb alone in 1998, whereas those with

coexistent TgAb and TPOAb positivity remained so in the following years. These findings suggest that the response of the immune system to iodine intake could be heterogeneous, possibly reflecting different immunological backgrounds (25). The high prevalence of TgAb alone in 1998 might reflect predominantly an autoimmune response, without significant involvement of the thyroid gland. Conversely, the coexistence of TgAb and TPOAb could be a clinical expression of more intense autoimmune processes, as suggested previously (31, 32). This may explain the greater percentage of subclinical hypothyroidism in the schoolgirls that was noted in 2001 (with predominant positivity for TgAb+ TPOAb) compared with 1998 (positivity for TgAb alone).

The significant difference in mean thyroid volume in the two groups is also noteworthy. We used standard ultrasound techniques (WHO/ICCIDD) (18) and applied a correction recommended by Zimmermann *et al.* (19) to calculate thyroid volume. The populations studied were comparable between the two surveys and were drawn from areas where UIE was adequate ($149.5 \pm 78.4 \mu\text{g/l}$ and $158.9 \pm 69.5 \mu\text{g/l}$) and previous prevalences of goitre were comparable. The fact that the mean UIE was not different between the two surveys is encouraging and suggests that the iodination programme has been effective. The lower prevalence of goitre in 2001 may have reflected a genuine reduction in thyroid volume with continuing exposure to iodine. Thyroid volume is a good long-term marker of the iodine status of a population (33–35). However, the effects on thyroid volume of other factors such as selenium intake (36) and nutritional status (37) cannot be totally excluded. A significantly lower BSA and LBM in the 1998 group would suggest that anthropometric variability did not account for the greater median thyroid volume (38) (Table 1), but would point to the involvement of relative 'malnutrition' as a risk factor. It was also noteworthy that the prevalence of goitre, corrected for BSA, was significantly greater in both studies compared with the prevalence of goitre corrected for age. The use of BSA would seem more appropriate in surveys of goitre prevalence, in populations that are nutritionally significantly different from the white western populations from which normative data have been formulated (39, 40).

In conclusion, our study demonstrates that, in Sri Lanka in 2001: (1) the prevalence of goitre and of thyroid autoimmunity were significantly lower than in 1998; (2) there was a modulation of thyroid autoimmunity compared with 1998, with a greater prevalence of TPOAb either alone or in combination with TgAb; (3) the occurrence of hypothyroidism was correlated with the presence of thyroid autoimmunity (but in 1998 a comparable percentage of Ab+ve and Ab-ve individuals developed hypothyroidism). These results would suggest a reversibility of the thyroid autoimmunity in some individuals and continuing and

perhaps more aggressive autoimmune activity in others during continued exposure to iodine. Further studies are needed to clarify the immunological determinants of these different outcomes.

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