CLINICAL STUDY

Thyroglobulin as a marker of iodine nutrition status in the general population

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Abstract

Objective: The iodine status of a population is traditionally evaluated by either urinary iodine (UI) excretion or by some measure of thyroid volume and the prevalence of goitre. In this prospective study of a mandatory iodization programme, we aimed to evaluate serum thyroglobulin (Tg) as a marker of iodine status in the population.

Methods: Two identical cross-sectional studies were performed before (1997–1998, $n = 4649$) and after (2004–2005, $n = 3570$) the initiation of the Danish iodization programme in two areas with mild and moderate iodine deficiency. Serum Tg was measured from blood samples. Thyroid volume was measured by ultrasonography.

Results: Before iodization, the median serum Tg was considerably higher in moderate than in mild iodine deficiency. Iodization led to a lower serum Tg in all examined age groups. The marked pre-iodization difference in Tg level between the regions was eliminated. The prevalence of Tg above the suggested reference limit (40 µg/l) decreased from 11.3 to 3.7% ($P < 0.0001$). Using bootstrapping, we demonstrated a higher efficacy of Tg than of thyroid volume to show a difference between pre- and post-iodization values.

Conclusion: We found serum Tg to be a suitable marker of iodine nutrition status in the population. The results may suggest that the Danish iodization programme has led to a sufficient iodine intake, even if the median UI excretion is still marginally low according to WHO criteria.

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Introduction

In measuring the iodine status of a population, the recommended measures are primarily urinary iodine (UI) concentration and thyroid size, either as goitre rate judged by palpation or as median thyroid volume and prevalence of thyroid enlargement measured by thyroid ultrasonography (US) (1). UI concentration reflects the immediate iodine status in the population reacting within days to a change in iodine intake and does not give any information about thyroid dysfunction. Thyroid volume, on the other hand, reflects not only the current iodine status, but also the iodine status in the preceding years, perhaps even lifetime iodine exposure (2).

Thyroglobulin (Tg), a thyroid-specific protein and precursor in the synthesis of thyroid hormones, has been suggested as a marker of the iodine status not only in the population reflecting thyroid abnormalities, but also iodine deficiency in the population as a whole

(3–8). It is measurable in serum in most individuals and the serum concentration increases with increasing thyroid mass, with inflammation of the thyroid and if the TSH receptors are stimulated (9).

The use of Tg is, however, impeded by inter-assay differences. Although attempts have been made to minimize this by international standardization (10, 11), considerable differences remain (9). Furthermore, it is not clear whether a concurrent assessment of Tg antibodies (TgAb), taking possible Tg assay interference into account, is necessary in a population context (12).

In a previous study, we demonstrated Tg as a sensitive marker of iodine deficiency with higher Tg values in an area with moderate iodine deficiency compared with an area with mild iodine deficiency (7). In this study, we aim to further describe Tg as a marker of iodine status on a population level with comparison to thyroid volume, and to evaluate the effect of the recent Danish iodization programme (13).

Subjects and methods

We performed two cross-sectional studies in two regions in Denmark with respectively mild and moderate iodine deficiency in relation to the introduction of a mandatory iodization programme.

The pre-iodization cross-sectional study

The first cross-section was carried out in 1997–1998 before any iodization of salt was allowed in Denmark. Two regions suspected to be mildly (Copenhagen) and moderately (Aalborg) iodine deficient were investigated. Persons in five age- and gender groups were invited from random lists: women aged 18–22 years, 25–30 years, 40–45 years, 60–65 years; and men aged 60–65 years. The age- and sex groups were chosen to obtain maximum information for the least cost. The selection- and invitation procedures as well as the study in general were described previously in detail (14). In all, 4649 participated (participation rate: 50.1%). Participants answered questionnaires regarding previous medical history and lifestyle factors, and gave blood and spot urinary samples.

The blood samples were stored at -20°C , and were subsequently analysed in a random order to mix them with respect to region, sex, age and season. In the first cross-section, serum Tg was analysed with immunoluminometric assays (LUMITEST, BRAHMS, Berlin, Germany) using a Stratec autoanalyzer (STRATEC-BIOMEDICAL SYSTEM AG, Birkenfeld, Germany). The assay had an effective working range of 1–500 $\mu\text{g/l}$.

TgAb were measured with an RIA (DYNAtest, BRAHMS) with a functional assay sensitivity below 20 kU/l. All assays included control samples at three levels at the start and end of each run. In 12 consecutive assays, the intra-assay and inter-assay variations (coefficients of variation, CV) for single determinations for controls with average Tg concentrations of 8.1, 45 and 154 $\mu\text{g/l}$ were (intra) 5.3, 2.8 and 4.2% and (inter) 6.8, 4.5 and 3.3%.

Non-fasting spot urinary samples were digested by alkaline ashing and analysed by the Ce/AS method for iodine contents as described previously (15, 16). Median UI concentration among individuals not taking iodine supplementation was 61 and 45 $\mu\text{g/l}$ respectively (crude median values: 68 and 53 $\mu\text{g/l}$), and the regions were considered mildly and moderately iodine deficient.

In all assays, the serum and urinary samples from the two regions and the different subsamples of the population were analysed in random order.

Finally, an US of the thyroid was performed using a Sonoline Versa Pro 7.5 MHz 70 mm linear transducer (Siemens, München, Germany), effective length 62 mm. Thyroid volume was calculated as maximal length $\times$ width $\times$ depth $\times \pi/6$ of each lobe. Thyroid enlargement was defined as a thyroid volume >18 ml for women

and 25 ml for men (17). The same two sonographers performed all the ultrasonographies. Their comparability is described previously (18).

The post-iodization cross-sectional study

Four years after initiation of the iodization programme, a new cross-sectional study was carried out in 2004–2005. Participants were matched by age and gender on a group level to participants in the pre-iodization study to enable direct comparison. The participation rate was 46.6% with 3570 subjects participating. The study has been described previously in detail (19). All procedures and apparatus were similar to the procedures used in the first cross-sectional study, and the ultrasonographies were performed by the same two ultrasonographers. Before the second study, a confirmatory inter-observer study was performed showing continued good correlation ($r=0.95$) in the determination of thyroid size (19).

In the second cross-section, serum Tg was analysed with an immunofluorescent assay (hTg KRYPTOR, BRAHMS). The detection limit was 0.17 ng/ml and the functional assay sensitivity was below 0.8 ng/ml (manufacturer's information). TgAb were measured with an immunofluorescent assay (anti-Tgn KRYPTOR, BRAHMS) with a detection limit of 10 U/ml and a functional assay sensitivity of 33 U/ml (manufacturer's information). In 31 consecutive assays, the intra-assay variations (CV) for single determinations for controls with average Tg concentrations of 1.7, 2.6 and 71 $\mu\text{g/l}$ were 4.5, 4.5 and 1.4%; and in 10 consecutive assays, the inter-assay variations for single determinations for controls with average Tg concentrations of 0.85, 1.8, 4.4 and 71 $\mu\text{g/l}$ were 14, 7.9, 4.2 and 4.6%.

The median iodine excretion values increased with 40–45 $\mu\text{g/l}$ after iodization and were now 108 $\mu\text{g/l}$ in Copenhagen and 93 $\mu\text{g/l}$ in Aalborg (20), making the areas iodine sufficient and mildly iodine deficient according to criteria outlined by WHO (1). After exclusion of subjects taking individual iodine supplementation, the figures were 99 and 86 $\mu\text{g/l}$ respectively.

Inter-assay difference of the Tg assays

Different methods were used for the measurement of Tg in the two cross-sections. Therefore, an inter-assay analysis ($n=48$) was performed by measuring Tg in the samples kept frozen from the first study with the new assay. There was a high correlation ($r=0.98$) between the methods used in the first and the second cross-section. However, the method used in the second cross-section measured, in general, lower values compared with the method used in the first cross-section. On average, the value was 87.2% of the expected value. This means that a possible fall in median Tg between the cross-sections might be overestimated. A Bland–Altman plot was constructed to illustrate the level of agreement

between the two methods. It demonstrated that the difference between the assays was most prominent for the highest value. For all subjects with a mean Tg value from the two assays above 30 µg/l ($n=12$), the assay used in the first cross-section measured a higher value than the assay used in the second cross-section. For subjects with mean Tg value below 30 µg/l ($n=36$), the differences were evenly distributed around zero making results from the assays more comparable. It is possible that the higher iodization level could lead to a changed folding of the Tg molecule, altering the result of the measurement. It could also not be excluded that the storage of samples from the first study had led to a minor loss of Tg immunoreactivity in the samples (11).

The study was approved by the regional Ethics Committee (jnr: VN 2004/13mch) and all participants gave written informed consent.

Statistical analysis

Data processing was done using SAS 9.1 + 9.2 statistical software (SAS, Cary, NC, USA). A non-parametric test (Mann–Whitney/Wilcoxon's test) was used comparing median serum Tg before and after iodization of salt, and in between the regions, as the distribution of serum Tg was log normal. Comparisons between frequencies of elevated Tg (above 40 µg/l, the recommended upper reference limit in iodine-replete populations (9)) were done using χ^2 -test.

As TgAb could be a source of Tg assay error (9), analyses were done both on the entire population and with exclusion of subjects with positive TgAb titre (>20 U/l).

In linear regression models investigating thyroid volume as an explanatory variable for Tg, possible confounding by region, sex, age, lifestyle (tobacco, alcohol and iodine supplementation) and body surface area was considered. Because of skewness, serum Tg

was logarithmically transformed for analyses in regression models.

To check whether an association between serum Tg and thyroid volume was stable across different levels of iodine intake in a population, an interaction term (thyroid volume $\times$ cross-section) was included. The level of significance was 0.05.

To investigate the efficacy of serum Tg compared with thyroid volume as a marker of iodine deficiency, we examined the 'number needed to investigate' to show a difference between the pre- and post-iodization values in our study. Although the analysis depends on the circumstances under which the study was performed (e.g. iodization level before and after), it offers a direct comparison of the sensitivity of the two methods. Using bootstrapping with replacement (21), 1000 subsamples of respectively 25, 50, 100, 200, 300, 400, 500, 600, 700 or 800 participants were drawn from our pre- and post-iodization populations. The pre- and post-iodization values of respectively serum Tg and thyroid volume were compared for each number of participants using Mann–Whitney/Wilcoxon's test. The percentage of comparisons, which showed a difference between the pre- and post-iodization subsample with a P value below 0.05, constituted the power of the study. We aimed at a power of 0.8.

Results

We found a lower median serum Tg in both genders and across all studied age groups after 4 years' iodization of salt (Table 1). The overall median serum Tg decreased from 10.9 to 8.7 µg/l ($P<0.001$) in the area with previous mild iodine deficiency and from 14.6 to 8.9 µg/l ($P<0.001$) in the area with formerly moderate iodine deficiency. Also, the inter-quartile range narrowed considerably after iodization (Fig. 1). After the

Table 1 Median serum thyroglobulin (Tg; µg/l) in two Danish regions ($n=8219$) with respectively formerly mild and formerly moderate iodine deficiency (ID) before and after the introduction of mandatory iodization of salt in Denmark. Values for Tg antibodies (TgAb)-negative subjects are stated in parentheses.

	Mild ID before iodization $n=2395$ ($n=2062$)	Formerly mild ID after iodization $n=1778$ ($n=1448$)	Before versus after P	Moderate ID before iodization $n=2188$ ($n=1866$)	Formerly moderate ID after iodization $n=1777$ ($n=1401$)	Before versus after P	Area versus area before iodization P	Area versus area after iodization P
18–22 years, women	8.6 (9.0)	7.6 (7.8)	<0.001 (<0.001)	12.1 (12.5)	7.8 (7.8)	<0.001 (<0.001)	<0.001 (<0.001)	0.41 (0.90)
25–30 years, women	9.9 (10.6)	7.5 (7.9)	<0.001 (<0.001)	12.6 (12.7)	7.7 (8.2)	<0.001 (<0.001)	<0.001 (<0.001)	0.09 (0.17)
40–45 years, women	12.4 (12.9)	9.6 (10.0)	<0.01 (<0.001)	18.3 (20.2)	9.4 (10.0)	<0.001 (<0.001)	<0.001 (<0.001)	0.97 (0.68)
60–65 years, women	14.2 (16.4)	11.0 (11.9)	<0.01 (<0.001)	18.5 (21.5)	10.3 (11.5)	<0.001 (<0.001)	<0.001 (<0.001)	0.78 (0.86)
60–65 years, men	10.8 (11.0)	9.0 (9.3)	<0.01 (<0.001)	14.3 (14.7)	10.3 (10.3)	<0.001 (<0.001)	<0.001 (<0.001)	0.03 (0.10)

Comparisons made using Mann–Whitney/Wilcoxon's test. Data on serum thyroglobulin were missing for 81 subjects.

Figure 1 Median serum thyroglobulin ($\mu\text{g/l}$) and inter-quartile range (whiskers) before ($n=4649$) and 4 years after ($n=3570$) iodization of salt in two regions of Denmark with mild and moderate iodine deficiency. Data on serum thyroglobulin were missing for 81 subjects.

iodization, there was only a difference in median Tg between the regions among the eldest women.

TgAb in serum can interfere with the Tg assay, and we performed all the analyses both on the entire population and after exclusion of individuals with a positive TgAb titre. As expected the values were higher, but otherwise there were only minor differences in the results (Tables 1 and 2) and the exclusion did not alter the conclusion.

The prevalence of subjects with Tg above $40 \mu\text{g/l}$ was 11.3% before iodization and 3.7% 4 years after iodization ($P<0.001$; Fig. 2).

When analysed in a univariate linear model, there was a highly significant association between individual serum Tg and thyroid volume ($r=0.31$, $P<0.0001$). The association remained ($P<0.0001$) when considered in multivariate models including other factors that might affect serum Tg (age, sex, region of inhabitancy, use of iodine containing dietary supplement and smoking status).

The studying of serum Tg and thyroid volume at US as markers of the shift in population iodine intake was conducted by analysing the number of participants needed to reach a significance level of 0.05 with a power

of 0.8. The analyses showed a higher efficacy of serum Tg than of thyroid volume. In the area with previous mild iodine deficiency, at least 300 participants were needed if serum Tg was used, whereas at least 400 participants were needed if thyroid volume was used (Fig. 3A). In the area with previous moderate iodine deficiency, the numbers needed for a demonstration of a difference were in general lower, as a more marked change in iodine nutrition status occurred. The difference between serum Tg and volume was pronounced, as twice as many participants ($n=200$) were needed to demonstrate a difference in thyroid volume as in serum Tg ($n=100$; Fig. 3B).

Discussion

We demonstrated a decline in median serum Tg in all investigated age groups after 4 years of mandatory iodization. The Danish iodization programme aims at relatively homogeneous distribution of iodine in the population with a modest fortification of household salt and salt used in the bread industry with 13 ppm iodine (13). This led to a fairly uniform increase in all groups with an average increase in median UI concentration of $45 \mu\text{g/l}$ in both the investigated regions (20).

The decline in serum Tg was most pronounced in the area with previous moderate iodine deficiency. After the iodization, there were only minor differences in serum Tg levels between the regions. The disappearance of a regional difference in all age groups indicates that the current iodine intake may be sufficient, despite a median UI concentration marginally below the limits recommended by the WHO (1). However, more studies are needed to further describe the effects of a marginally low iodine intake, whether negative or positive.

We previously examined the relationship between thyroid volume and iodine nutrition level in these subjects and found a comparable association. The equalization of thyroid volume between the regions was, however, only complete for participants below 45 years of age (19). This suggests that Tg is a better marker of the present iodine nutrition status in a population than thyroid volume, which additionally reflects the consequences of a previous iodine intake, at least in populations with decades of low iodine intake.

High levels of serum Tg were demonstrated in endemic goitre areas already in the seventies (22).

Table 2 Inter-quartile range and prevalence of elevated thyroglobulin (Tg) before and after mandatory iodization of salt in two regions with mild and moderate iodine deficiency. Values for Tg antibodies (TgAb)-negative subjects are stated in parentheses.

		<i>n</i>	Median ($\mu\text{g/l}$)	Inter-quartile range ($\mu\text{g/l}$)	Tg > $40 \mu\text{g/l}$ (%)
Mild	Before	2395 (2062)	10.9 (11.5)	5.6–19.8 (6.2–20.2)	7.6 (7.5)
Formerly mild	After	1778 (1448)	8.7 (9.1)	5.3–13.7 (5.8–13.9)	3.0 (3.0)
Moderate	Before	2188 (1866)	14.6 (15.4)	7.0–28.9 (7.8–29.8)	15.0 (15.4)
Formerly moderate	After	1777 (1401)	8.9 (9.3)	5.5–15.4 (5.8–15.7)	4.4 (4.1)

Figure 2 The prevalence of elevated serum thyroglobulin in three classes before ($n=4649$) and 4 years after ($n=3570$) iodization of salt in two regions of Denmark with mild (A) and moderate (B) iodine deficiency. Data on serum thyroglobulin were missing for 81 subjects.

Subsequently, lowering effect of iodine injections on Tg was demonstrated among subjects in long-term, severe iodine-deficient areas (3, 4). A double-blinded randomized study of the effect of a single oral dose of iodized oil in children living in a moderate to severe iodine-deficient region concluded that serum Tg (together with UI concentration) was the most appropriate measure for iodine status and thyroid function (23). Elevated levels of serum Tg have also been demonstrated in areas with only mild to moderate iodine deficiency, with differences in Tg level according to the iodine nutrition level in the population (7, 24, 25).

Serum Tg can be elevated due to large thyroid mass, thyroid inflammation or when TSH stimulates the thyroid. The generally increased thyroid mass found under iodine-deficient conditions most likely explains part of the elevation of serum Tg. However, thyroid volume alone does not explain the elevation of serum

Tg found in iodine-deficient regions; elevated Tg is also found among subjects without goitre living in iodine-deficient areas (22). Also, serum Tg has been demonstrated to be associated with various measures of iodine intake (8). This suggests Tg to be associated with the iodine nutrition status in the population, independently from the association with thyroid volume. As opposed to UI concentration (26, 27), serum Tg shows only little day-to-day variation (28). Even though it did not reach significance in a relatively small Hungarian sample, a trend towards more individuals with high Tg level in areas with iodine abundance has been demonstrated (29). In a large Chinese study, the median Tg did not differ between three regions with mild iodine deficiency, iodine adequacy and more than adequate iodine intake respectively (30). However, the iodine excess in the

Figure 3 The power to show a significant ($P<0.05$) difference in serum thyroglobulin (solid line) and thyroid volume (broken line) with increasing number of participants in the study population drawn from two regions in Denmark, which were respectively mildly (A) and moderately (B) iodine deficient before iodization of salt. Results are from a bootstrapping using Mann–Whitney/Wilcoxon's test for comparison of pre- and post-iodization values.

Chinese study was not as pronounced as in the Hungarian study.

Other factors may also attribute to the lower level of Tg. It has been suggested that the higher iodine intake may lead to less oxidative stress and thereby a lower Tg level. A lower TSH level cannot explain the lower levels of Tg, as we (as expected) demonstrated a higher median TSH after iodization of salt in this population (1.51 vs 1.30 mU/l before iodization (31)).

An attempt has been made to establish a Tg reference interval for iodine sufficiency among children (32), but no limits exist for adults. A median serum Tg below 10 µg/l in the population has been suggested as an indication of iodine sufficiency and was previously included in the WHO criteria for iodine deficiency (32). It was, however, removed from the 2001 WHO guidelines (33). Our results support a median serum Tg in the population <10 µg/l as a marker of iodine sufficiency, at least employing the Tg methods of the present study.

By the use of bootstrapping, we studied the efficacy of serum Tg compared with thyroid volume at US as biological markers of population iodine intake. This method enabled us to give an estimate of the number needed to investigate for the demonstration of a difference with a given power. The method depends entirely on our population and the numbers demonstrated cannot be applied to other populations. However, we solely used this method to compare two methods of studying markers of iodine deficiency in a given population. We demonstrated a higher efficacy of serum Tg than of thyroid volume in a population shifting from mild/moderate iodine deficiency to borderline iodine sufficiency, if the purpose is solely to monitor iodine deficiency and its general effect on the thyroid. Thyroid US has, on the other hand, other advantages. It gives a picture of thyroid size and enables description of thyroid structure and existence of thyroid nodules.

With the development of a stable dried whole-blood spot Tg assay (34), the measurement of Tg has become possible even in remote areas. There is an ongoing discussion on the necessity to perform a concurrent TgAb measurement to enable control of assay interference caused by TgAb. Exclusion of participants with positive TgAb titre did not alter our conclusions on the relationship between iodine nutrition level, thyroid volume and Tg. This might suggest that the measurement of TgAb is unnecessary when Tg is used in a population context, although this may be method-dependent (9).

The main strength of our study is the design with two comparable cross-sectional studies using the same procedures and apparatus before and after iodization of salt. This allows a direct comparison of the studies and thereby an evaluation of the effect of iodization. However, there was a change in method by which serum Tg was measured. The inter-assay comparison showed that the new method measured a lower serum

Tg than the former in subjects with high serum Tg. With the predominantly lower value measured with the new method, an overestimation of the effect of the higher iodization level on serum Tg has most likely occurred. However, the difference in method can not explain all the difference, e.g. the differentiated effects in the age groups and regions and the narrowing of the range of serum Tg. Also, the main part of Tg values in population studies is found in the lower range of Tg values, allowing the demonstrated difference in methods less impact.

In conclusion, we found serum Tg to be a suitable marker of the recent iodine status in a population. Apparently, a median serum Tg around 10 µg/l in a population might be used to identify iodine sufficiency.

Declaration of interest

There are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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