CLINICAL STUDY

Serum TSH and serum thyroid peroxidase antibody fluctuate in parallel and high urinary iodine excretion predicts subsequent thyroid failure in a 1-year study of patients with untreated subclinical hypothyroidism

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Abstract

Objective: To explore the possibility of predicting decline or improvement in thyroid function over 1 year, and to investigate the correlations of serum TSH (s-TSH) with hypothyroidism-related symptoms and signs, serum thyroid peroxidase antibody (s-TPO-Ab) and urinary iodine excretion in individual patients with untreated subclinical hypothyroidism (SH).

Design: Monthly repeated measurement study without intervention.

Methods: Twenty-one patients without former thyroid disease who had been identified with s-TSH between 5 and 12 mU/l and normal serum thyroxine (s-T₄) at two occasions were enrolled. Subsequently, 13 monthly measurements of s-TSH, hypothyroidism-related symptoms and signs, serum free T₄, s-TPO-Ab and urinary iodine excretion were performed.

Results: Over the study year, s-TSH increased significantly in 5 patients, 16 had unchanged s-TSH, whereas none improved. From clinical and biochemical inclusion data, it was not possible to predict who would later increase in s-TSH. In individual patients, a highly significant correlation between s-TSH and s-TPO-Ab was found ($r=0.37$, $P<0.0001$) and also between s-TSH and urinary iodine excretion ($r=0.14$, $P=0.034$). No correlation between s-TSH and clinical symptoms and signs was observed. Time shift showed best correlation between s-TSH and s-TPO-Ab measured at the same time point, whereas urinary iodine excretion correlated best to s-TSH and s-TPO-Ab obtained 1 month later.

Conclusion: At the time of inclusion, it was not possible to identify the 24% of SH patients who would show deterioration in thyroid function over the following year. Impairment in thyroid function varied in parallel with thyroid autoimmunity, whereas high urinary iodine excretion predicted high s-TSH and s-TPO-Ab 1 month later.

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Introduction

Subclinical hypothyroidism (SH) is a condition with serum thyrotrophin (TSH) above the upper reference limit for the assay and serum thyroxine (T₄) within the reference range. SH is frequent in most populations studied (1–5), but the underlying abnormalities responsible for development and variation in thyroid function in SH are poorly understood. SH is often linked to thyroid autoimmunity, and an association between elevated TSH and the presence of thyroid peroxidase antibody (TPO-Ab) in serum has been found in several cross-sectional studies (3, 4, 6–8). During long-term follow-up with large intervals between clinical controls, both high TSH and the presence of TPO-Ab are associated with a high risk of future progression to overt hypothyroidism (9) and some patients will normalise in thyroid function (10). Short-term changes in TSH and TPO-Ab in serum in patients with SH on the other hand are largely unknown.

In intervention studies, it has been found that excessive iodine intake may worsen hypothyroidism in individuals with autoimmune thyroiditis (11, 12), and it may be speculated that variation in dietary iodine intake may influence thyroid function in patients with SH.

We studied the course of thyroid function and hypothyroid symptoms and signs in patients with SH, and how this was associated with variations in thyroid autoimmunity and iodine intake by monthly assessments during 1 year. We also investigated the possibility of predicting at the time of inclusion a decline or improvement in thyroid function over the subsequent year.

Patients and methods

After we had informed general practitioners in our area about the investigation, 44 patients with the result of a single TSH measurement in the interval 5–12 mU/l and a

normal serum total T_4 as controlled by their general practitioner were referred to us and contacted. After information about the study had been given, 34 patients were willing to participate and they were subsequently examined in our investigational unit. Thyroid function tests were performed 3 months after the initial test and patients still having TSH in the 5–12 mU/l interval and normal T_4 were included in the study. Exclusion criteria were: former thyroid disease, age below 18 or above 80 years, change in any type of medication during the last 3 months, requirement of medication with influence on thyroid function, diseases with potential influence on thyroid function or pregnancy within the last 12 months. In 10 patients, thyroid function had normalised during the 'run-in' 3 months; thus, 21 participants, all Danish Caucasians were included during the period May 2004 to July 2005. The patients were recruited throughout the year and investigated according to inclusion date, in order to abolish any seasonal effect. All participants lived in or close to Aalborg, Jutland, Denmark, an area with previously moderately low iodine intake, but with effective salt iodisation since 2001 (13).

After inclusion, participants were investigated monthly on a total of 13 occasions. The interval between investigations was on average 37 days. The patients attended the investigational sessions after an overnight fast. One patient (patient 21) became progressively and overtly hypothyroid and, according to protocol, received $L-T_4$ treatment after 4 months. This participant was excluded from the calculations as indicated.

Hypothyroid symptoms and signs were evaluated as described by Zulewski (14). This clinical evaluation included seven thyroid-related symptoms and five signs that are summarised in a total score. Higher score indicates more thyroid-related symptoms and signs. Patients were weighed (digital weight, SECA, Kolding, Denmark) in light clothing and height measured with a stadiometer. Thyroid volume was estimated ultrasonographically, by the use of a Siemens Sonoline Versa Pro (Siemens, Munich, Germany) and a 70 mm 7.5 MHz linear transducer as described in detail by Knudsen *et al.* (15). All ultrasound measurements were performed using the same experienced operator. Blood samples were drawn between 0900 and 1200 h after the patients had been in supine position for 30 min and after brief venous occlusion. Samples were centrifuged at 1200 *g* and frozen at -20°C shortly after the investigational session. Urine samples were fasting spot urine.

Assays

All assays performed on the study samples were done as single batch operations of samples in random order using an Electro-Chemi Luminescence ImmunoAssay method on a Modular Analytics E170 (Roche). Assay characteristics given by the manufacturer with detection limits and normal ranges were as follows: TSH, 0.005 mU/l, 0.27–4.2 mU/l and fT_4 , 0.3 pmol/l, 12–22 pmol/l. Intra-assay coefficient of variation (CV) for the TSH and

fT_4 assays were 4.5 and 3.4%. Total T_4 for the inclusion was measured on the same apparatus, with lower detection limit of 5.4 nmol/l, laboratory reference range of 60–140 nmol/l and total precision CV of 3.7%. TPO-Ab and thyroglobulin antibody (Tg-Ab) were measured by anti-TPO and anti-Tg KRYPTOR (BRAHMS, Henningsdorf, Germany). Analytical sensitivity for TPO-Ab was 10 U/ml, intra-assay CV% 4.2 and inter-assay CV% 9.7. Analytical sensitivity for Tg-Ab was 10 U/ml, intra-assay CV% (for the range 55.4–502 U/ml) 4.7 and inter-assay CV% (for the range 69.3–517 U/ml) 6.5, as stated by the manufacturer. Urinary iodine was determined by the Ceri/Arsenium method after alkaline ashing (16, 17) and expressed as estimated 24-h urinary iodine excretion (μg ; 24-h U-iodine excretion), estimated from concentrations of iodine and creatinine in the spot urine samples as described by Knudsen *et al.* (18). For the estimations, sex- and age-specific 24-h urinary creatinine excretions as provided by Kesteloots *et al.* (19) were used. Urinary creatinine was measured by a kinetic Jaffé method. All investigational sessions except one were performed using the same investigator (J K).

All participants signed an informed consent form before entering the study, and the study was approved by the Regional Ethics Committee in North-Jutland and Viborg County, Denmark.

Statistical evaluation

Linear regression was used for estimation of trends over time. Differences in trends between two groups were investigated with the use of summary measure as described by Matthew & Altman (20). Individual regression coefficients of variables versus time were used as summary measures. For comparison between groups, Mann–Whitney and Fisher's exact tests were used as appropriate. Within-person correlation and between-person correlation were calculated as suggested by Bland & Altman (21). Pearson correlation coefficient was used for time-shifted correlation analyses. Differences were considered significant at $P < 0.05$. Statistical Package for Social Sciences (SPSS versus 11.0, Chicago, IL, USA) and Excel 2003 (Microsoft Corp.) were used for statistical calculations.

Results

Patients' characteristics at first investigation after inclusion are shown in Table 1. TSH, fT_4 and TPO-Ab displayed significant trends, over the next 12 months when analysed for the entire group of patients. TSH increased linearly with time ($P = 0.002$), whereas fT_4 decreased ($P = 0.002$) and TPO-Ab increased linearly ($P = 0.002$). Hypothyroid symptom score showed no time dependency ($P = 0.90$), neither did 24-h U-iodine excretion ($P = 0.96$). On the basis of linear models, time to reach a 10% difference from

Table 1 Patient characteristics at the first investigation after inclusion.

	All patients	Positive trend in TSH	No trend in TSH	P value*
No. of patients ^a	21	5	16	
Sex (male/female) ^a	2/19	0/5	2/14	1.00
Tobacco (yes/no) ^a	4/17	0/5	4/12	0.53
BMI (kg/m ²)	26.5 (25–33)	25.7 (24–26)	27.4 (25–35)	0.10
Age (years)	57 (51–66)	55 (34–62)	57 (52–67)	0.25
TSH (mU/l)	6.5 (4.9–8.0)	7.8 (4.7–8.6)	6.2 (5.0–8.0)	0.62
fT ₄ (pmol/l)	13.7 (12.6–14.5)	13.5 (12.6–14.1)	14.2 (12.6–14.6)	0.48
TPO-Ab (10 ³ × kU/l)	1.75 (0.76–10.5)	1.00 (0.31–14.0)	4.90 (9.10–11.1)	0.46
Tg-Ab (kU/l)	115 (7.5–280)	115 (7.5–362)	129 (16–266)	0.87
24-h U-iodine (μg)	102 (82–147)	102 (67–134)	105 (83–153)	0.41
Thyroid volume (ml)	9.5 (7.0–11.2)	7.6 (5.7–9.9)	9.7 (7.4–12.8)	0.08
Hypothyroid score	2 (1–3)	1 (0–1)	2 (1.25–3)	0.97

Data are shown for all patients with subclinical hypothyroidism and for groups according to trend in s-TSH over the following 1 year. BMI, body mass index; TSH, thyroid stimulating hormone; fT₄, free thyroxine; TPO-Ab, thyroid peroxidase antibody; Tg-Ab, thyroglobulin antibody; U-iodine, urinary iodine

*P value for comparison of groups with and without trend in TSH using Fisher's exact or Mann-Whitney tests as appropriate.

^aNumbers; all other values are medians (interquartile range).

baseline was 7.8 months for TSH and 15.7 months for fT₄. Time for TPO-Ab to reach a 10% difference was 6.6 months, comparable with that for TSH.

Subgroups of patients with different course in TSH

In the individual patient, TSH might theoretically decrease, increase or remain unchanged with time. When trend in TSH over time for each patient was estimated by linear regression, none of the patients had a significant decrease in TSH. Five patients (patient number 10, 13, 17, 19 and 21) had a significant increase and 16 patients had no statistical significant change in TSH during the study period. Figure 1 displays the average TSH, fT₄, hypothyroid score and 24-h U-iodine excretion over 1 year in patients stratified according to trend in TSH. The group of patients with an increase in TSH had a more pronounced decrease in fT₄ (−0.19 pmol/l per month versus −0.06 pmol/l per month, $P=0.03$), and increase in hypothyroid score (0.14 score/month versus −0.018 score/month, $P=0.014$) and 24-h U-iodine excretion (5.3 μg/24 h per month versus −2.5 μg/24 h per month, $P=0.011$) compared with the group with no change in TSH. On the other hand, we observed no differences with regards to changes in TPO-Ab (2.7 kU/l per month versus 4.6 kU/l per month, $P=0.113$) and Tg-Ab (262.1 kU/l per month versus 76.2 kU/l per month, $P=0.186$) between the two trend groups.

The possibility of forecasting at the time of inclusion which patient would have an increase in TSH during the subsequent 12 months of follow-up was investigated as shown in Table 1. No significant differences were observed between the two groups of patients at the time of inclusion.

Association between thyroid insufficiency and antibody level in the individual patient

The associations between TSH and hypothyroid score, fT₄, TPO-Ab, Tg-Ab or 24-h U-iodine excretion in the individual

patient were evaluated by within-person correlation analyses as shown in Table 2. As expected strong correlations were observed between TSH and fT₄, but also between TSH and TPO-Ab, whereas a weaker correlation was found between TSH and 24-h U-iodine excretion. Hypothyroid score and Tg-Ab did not correlate to TSH (Table 2). The parallel fluctuations between TSH and TPO-Ab in the individual patients are illustrated in Fig. 2.

Time sequence of changes in TSH, TPO-Ab and 24-h U-iodine excretion

We studied if correlations between some variables were dependent on whether the variables were in phase or not. This was done by calculating coefficients of correlations with time shifts between the variables. Figure 3 shows mean of individual Pearson correlation coefficients with no time shifts between variables (abscissa value 0, indicated by vertical dotted line) and when variables were up to 6 months out of phase. As seen in the upper panel of Fig. 3, a positive correlation between TPO-Ab and TSH was seen over a broad time interval from TPO-Ab measured 2 months before to 1 month after TSH, whereas the only substantial correlation between 24-h U-iodine excretion and TSH was obtained using the 24-h U-iodine excretion value taken 1 month before the TSH value (middle panel). Similarly, Fig. 3, lower panel, shows that 24-h U-iodine excretion obtained 1 month before the TPO-Ab value gave the highest, and positive, mean correlation value.

Discussion

In this cohort of patients with untreated SH followed closely for 1 year, none improved in thyroid function whereas 24% showed deterioration, with a significant increase in TSH. Several investigations have carried out follow-up studies on patients with SH (10, 22–26), but none of these studies used as short and regular control intervals as the present study.

Figure 1 Serum TSH, fT₄, hypothyroid score and 24-h urinary iodine excretion measured 13 times over 1 year in patients with increasing TSH (+TSH trend, *n*=4, dotted line with triangles) and in patients without stable TSH values (–TSH trend, *n*=16, solid line with circles). Values are mean (±S.E.M.). The patient who developed profound overt hypothyroidism and started therapy was excluded from calculations. *P* values of differences between the groups were: fT₄, *P*=0.03; hypothyroid score, *P*=0.01 and urinary iodine excretion, *P*=0.01.

Four-year follow-up of SH patients from the Wickham study showed that 8 out of 48 developed OH. Seven out of these eight patients had both microsomal antibodies and elevated TSH at the first investigation, but levels were not different in the individuals who developed OH compared with the rest of the cohort with SH (23). Dies & Iglesias (26) followed 107 SH patients for a mean of 32 months. Twenty-eight required L-T₄ treatment during follow-up and 40 normalised thyroid function. Initial TSH level was predictive of future decline in thyroid function in this cohort. One (5%) of the patients in the present study developed requirement of L-T₄ treatment and 24% shared a decline in thyroid function over the relatively short time span of 12 months. At the time

of inclusion, it was not possible to predict which patients would have a further decrease in thyroid function.

The association between TSH and TPO-Ab has been studied in a number of cross-sectional studies (3, 4, 6–8). In the DanThyr cohort studied by Pedersen *et al.* (6), high TPO-Ab was found to associate with high TSH. The association was present at TSH above 2.5 mU/l. In patients with untreated SH, within-person correlation between TSH and TPO-Ab has not previously been evaluated. When our patients were observed as a cohort, a positive but insignificant correlation (*r*=0.40, *P*=0.08) was found between TSH and TPO-Ab. On the other hand, when individual patients were studied over 1 year a highly

Table 2 Within-person correlations between serum thyrotrophin (s-TSH) and free thyroxine (fT₄), hypothyroid score, thyroid peroxidase antibody (TPO-Ab), thyroglobulin antibody (Tg-Ab) and 24-h urinary iodine excretion (U-iodine).

Serum TSH versus	Correlation coefficient <i>r</i> ^a	<i>P</i> value
fT ₄	–0.43	<0.001
TPO-Ab	0.37	<0.001
Tg-Ab	0.09	0.15
U-iodine	0.14	0.034
Hypothyroid score	–0.017	0.79

^aCalculated as: $\sqrt{\text{predictor sum of squares}/(\text{predictor sum of squares} + \text{residual sum of squares})}$, from multiple regression ANOVA table, as described by Bland & Altman (21). fT₄, free thyroxine; TPO-Ab, thyroid peroxidase antibody; Tg-Ab, thyroglobulin antibody; U-iodine, urinary iodine.

Figure 2 Thirteen monthly s-TSH values (dotted lines, triangles) and Serum thyroid peroxidase antibody (s-TPO-Ab) values (solid lines, circles) for the 18 patients with measurable TPO-Ab (the number in the box corresponds to patient number). Patient 21 was started on L-T₄ treatment after the fourth investigation.

Figure 3 Mean (± 2 S.E.M.) of individual Pearson correlation coefficients between the variables indicated. Coefficients were calculated with no time shifts between variables (abscissa value 0, indicated by vertical dotted line) and when variables were up to 6 months out of phase. Markers to the left of the vertical dotted line represent correlations between the variables when the first variables were taken 1–6 months before the second. The right side of the vertical dotted line is the correlation when the first variables were taken 1–6 months after. Error bars not crossing the horizontal zero line indicate that the mean values are significantly different from 0 ($P < 0.05$).

significant positive within-person correlation between TSH and TPO-Ab was observed. Thus, in the individual patient, variation in thyroid failure paralleled activity in thyroid autoimmunity.

High iodine intake may lead to thyroid failure (5). Whether the major mechanism is iodine triggering of thyroid autoimmunity or whether it is a direct inhibitory effect on thyroid hormone production and secretion has been discussed for several years. Bournaud & Orgiazzi (27) reviewed the topic, and concluded that from a population-based perspective iodine sufficiency favours occurrence of autoimmune thyroid disease, but the effect of iodine administration appears to depend on several factors, such as iodine status of the recipient, dose of administered iodine, degree of stimulation of the thyroid gland and genetic factors. Reinhardt *et al.* (11) gave, in a randomised controlled study, a small amount of iodine to individuals with euthyroid Hashimoto's thyroiditis. The study subjects lived in a mildly iodine-deficient area. Seven persons in the treatment group ($n=40$) and one in the control group ($n=43$) developed SH. No change in TPO-Ab levels were observed, suggesting a direct inhibitory effect of iodine on thyroid hormone production/secretion. In our study, correlations between TSH and TPO-Ab or 24-h U-iodine excretion indicated that timing of events was important. The positive correlation between TSH and TPO-Ab took place over several months. On the other hand, 24-h U-iodine excretion correlated best to TSH values obtained 1 month later and also to TPO-Ab values obtained 1 month after 24-h U-iodine excretion. Our findings may indicate an additive effect of minor dietary increases in iodine intake and a surge in thyroid autoimmunity on thyroid failure in patients with SH.

Limitations

The patients studied by us were included after SH had been confirmed on two occasions 3 months apart, whereas many other studies have included patients with only one set of abnormal thyroid function tests (10, 22, 23, 25). This is probably the reason that none of our patients improved in thyroid function over the subsequent year, whereas this has been frequent in other studies (10, 22, 23). The urinary iodine excretion of the patients in the present study was still borderline low despite the Danish salt iodisation programme that has been effective since 2001 (13). Fasting spot urine samples, as were used in the present study, are known to have lower iodine content than non-fasting spot samples (28). Fasting urinary iodine concentration may represent a broader measure of the iodine status over a period than non-fasting concentrations, as the iodine concentration in a non-fasting urine sample to a high degree reflects the iodine content of the preceding meal.

Conclusion

In the individual patient, it was not possible to predict at the time of inclusion, whether there would be progression

in thyroid failure over the coming year. When studied closely, considerable fluctuations were found in thyroid function in patients with SH. In the individual patients, thyroid failure correlated closely to thyroid autoimmune activity but also to iodine intake during the preceding period. Iodine intake and thyroid autoimmunity may act synergistically to determine short-term variation in thyroid function in patients with SH.

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