Serum Thyroglobulin as a Risk Factor for Thyroid Carcinoma

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Samples from a biological serum bank taken up to 23 years prior to diagnosis of thyroid carcinoma were analysed for human thyroglobulin, thyroid-stimulating hormone and thyroxin. After exclusions, the final study material consisted of 59 cases of papillary and follicular carcinomas. These cases were compared with 164 controls, matched for sex, age and time of sample taking. The most interesting finding was that concentrations of thyroglobulin in serum were abnormally elevated in cases compared with controls, equal to or above 30 μg/L, with odds ratio 7.0 (CI 3.1–15.7). This elevation of serum thyroglobulin occurred in 44% of the carcinoma cases. Sensitivity was around 50 for measurements taken up to 15 years prior to diagnosis, but 21 when the interval was over 15 years. Specificity was 89. No differences were found between cases and controls in values for thyroid-stimulating hormone and thyroxin.

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Thyroglobulin, a prohormone or a precursor of the thyroid hormones, is produced and secreted by the follicular cells into the colloid of the follicular lumen of the thyroid gland. There, it is found in high concentrations but it is also demonstrable in the serum of most normal persons. Elevated blood concentrations of thyroglobulin can be found both in benign conditions such as thyroiditis or hyperthyroidism and in malignant conditions such as papillary or follicular thyroid carcinoma.

In 1988 Thoresen et al. (1) reported a case–control study showing that serum samples from patients subsequently diagnosed with thyroid cancer contained significantly increased concentrations of thyroglobulin. In a prospective study of children exposed to therapeutic head and neck irradiation for benign conditions, Schneider et al. (2) showed that some had elevated levels of thyroglobulin, which eventually led to a clinical disease some years later. Numerous studies have shown that although serum thyroglobulin (s-Tg) can be a valuable marker of recurrence in patients treated for thyroid carcinoma (3, 4), it has not been accepted as a screening test for thyroid malignancy, because of its low specificity. Serum thyroglobulin does not differentiate between benign and malignant thyroid disease, a disadvantage accentuated by the relatively low prevalence of thyroid carcinoma. In thyroidectomized patients the concentration of s-Tg is strongly influenced by the serum level of the thyroid-stimulating hormone (TSH) (5).

The purpose of the present study was to investigate further how frequently and by how much s-Tg might be increased in serum samples taken from Icelandic patients, later diagnosed with thyroid cancer.

MATERIAL AND METHODS

The Icelandic study of risk factors for cardiovascular diseases, often called the Reykjavik Study, was started in 1967 (6). Those invited to participate in the Reykjavik study in December 1, 1966 were mostly legal residents of the metropolitan area of Reykjavik. A small group of a rural population and young people were also invited. The total population studied amounted to 31528. The participants were born between 1907 and 1935 and were divided into five groups, by date of birth. The study was also divided into five stages, according to time of examination. The first group, stage I, started in 1967, and the fifth stage ended in 1991. The first group, comprising 6055 subjects, was invited to participate five times, along with other new groups. The mean response rate was 73%. At the visit a questionnaire was completed and biochemical measurements were carried out. Serum samples from each visit were stored at −20°C. The Icelandic Cancer Registry was initiated by the Icelandic Cancer Society in 1954 and has been operated by that society since that time (7). The registry covers the entire population of Iceland and records incidences of carcinoma by site. The registry receives infor-

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mation from all pathology and cytology laboratories throughout the country, as well as from hospitals, general practitioners and specialists. Complete follow-up of the study population, as well as population-based cancer registration, is greatly facilitated by The Icelandic National Registry, which has been in operation since 1952. In this registry, every member of the Icelandic population has a unique identification number, which is used by the Cancer Registry for linkages with the study population.

All histology slides from the thyroid tumours were reviewed and the tumours were classified according to the World Health Organization (WHO) classification system. Routine haematoxylin and eosin-stained slides were used for this classification.

Human serum thyroglobulin (s-Tg), TSH and thyroxin (T4) were assayed with reagents (DELFIA) from Wallac Oy, Turku, Finland. The s-Tg assay is a solid phase, two-site immunofluorometric assay based on a direct sandwich technique, in which two monoclonal antibodies are directed against two different epitopes on the human thyroglobulin molecule. Autoantibodies to thyroglobulin in serum will tend to block the binding of the specific monoclonal antibodies and, for the immunoradiometric assays, may cause falsely low or zero values. This can be tested to a great extent by adding s-Tg to the specimens and assessing the recovery. This was done in the present investigation and the results discarded if antibodies were likely to be present, as indicated by a recovery of less than 80%. The imprecision (CV) of the assay in our hands was similar to that given by the manufacturer, or 3.0% within and about 5.0% between assays. Detection limit was 0.2 μg/L, when defined as two standard deviations above the mean zero standard (blank) readings. The normal reference range was 2.0–30 μg/L for both sexes.

The TSH assay, a solid phase two-site immunofluorometric assay, was based on the direct sandwich technique, in which two monoclonal antibodies were directed each against two separate antigenic determinants (epitopes) on the TSH molecule. The manufacturer states the detection limit of the assay to be typically better than 5 nmol/L (0.39 μg/dL). The TSH assay had about 5% and 7% imprecision within and between runs, respectively, with a detection limit of 0.03 mU/L and normal reference range of 0.24–2.90 mU/L.

The AutoDELFIA T4 assay was a time-resolved solid phase immunofluoroassay. The imprecision of the T4 assay (%CV) was < 4.0 for both within and between runs with a normal reference range of 55–150 nmol/L.

Eighty individuals were identified as having thyroid malignant tumours and also as having attended the Reykjavik study before diagnosis of the thyroid tumours between 1967 and 1996, both years included. For each cancer patient three controls were selected from the serum bank, matched for sex, age and calendar year of blood sampling. Samples from cases and controls were randomly numbered and distributed for assay purposes and the codes were not broken until all analytical results had been reported.

Cases grouped according to histology, sex and extent of disease are listed in Table 1. As s-Tg is connected to differentiated thyroid cancer (papillary and follicular), our main analysis is concentrated on this group, excluding incidental papillary cancer. Six of these cases were excluded because of autoantibodies in serum, therefore 59 cases are included in the final study group. Each case had 3 controls but 13 had autoantibodies and were excluded, leaving 164 controls. Seventeen cases had more than one visit to the Reykjavik study and therefore we had 2–4 s-Tg measurements at different times. Altogether, there were 27 additional measurements in these cases.

Statistical methods

Conditional logistic regression was performed where the dependent variable was case/control and independent variable s-Tg positive (≥ 30 μg/L) or not. The s-Tg value was also tried as a predictor. Linear regression was applied for cases (all measurements) to test the dependence of the s-Tg value on gender, age and time until diagnosis of cases.

RESULTS

In Table 2 we show the separation of cases according to histologic type, whether the serum thyroglobulin value is increased and the presence of autoantibodies in serum. Thyroglobulin values for the anaplastic cancers were low (4.4, 7.0). For the cases incidentally found at autopsy, the mean value was 16.3 (range 5.0–34.7).

Table 1

Cases according to sex, histology and extent of disease

	Total	Sex		Without metastasis	Lymph node metastasis	Distant metastasis
		Male	Female			
Papillary	56	18	38	41	13	2
Follicular	9	6	3	8	1	0
Medullary	1	1	0	0	1	0
Anaplastic	2	1	1	0	2	0
Papillary incidental	12	7	5	12	0	0
Total	80	33	47	61	17	2

Table 2

Cases according to histology and thyroglobulin values and presence of autoantibodies in serum

	Thyroglobulin value		Autoantibodies in serum
	Normal	Elevated	
Papillary	30	21	5
Follicular	3	5	1
Anaplastic	2	0	0
Papillary incidental	30		
Medullary			1

Table 3

Results of conditional logistic regression analysis. Cases (papillary and follicular) and controls with serum thyroglobulin values within and above reference limits

	Total	s-Tg values μg/L		Odds ratio from conditional logistic regression (95% CI)
		<30/30	≥30/30	
Cases	59	33	26	7.0 CI (3.1–15.7)
Controls	164	146	18	

Thyroxin (T4) and TSH values were measured both for cases and for controls. Mean TSH values for cases and for controls were 2.4 (SD 1.6) and 2.7 (SD 3.1), respectively. One case and three controls had suppressed TSH values (< 0.1). The mean T4 values for cases and for controls were 97.7 (SD 18.6) and 99.1 (SD 23.1), respectively. The lowest T4 value for a case was 59 and for a control 39.

The results of the conditional logistic regression analysis are reported in Table 3. The odds ratio indicates that persons with elevated serum thyroglobulin are at a sevenfold risk of being subsequently diagnosed with thyroid cancer, papillary or follicular. The s-Tg value was found to be slightly better than s-Tg positivity as a predictor. Table 3 also shows that 26 out of 59 cases (44%) had s-Tg values equal to or more than 30 μg/L, whereas 18 out of 164 controls (11%) had s-Tg values equal to or above 30 μg/L.

Table 5

Cases, percentages and mean s-Tg values for all measurements of cases according to time before diagnosis

Time until diagnosis	No. of cases	Percentages	Mean s-Tg values
Years			
0.0–4.9	23	57	152
5.0–9.9	25	68	125
10.0–14.9	22	36	41
15.0–19.9	16	25	25
Total	86	49	92

For papillary carcinomas this was 41% but for follicular carcinoma the proportion was 63%.

S-Tg values of cases were found to increase with decreasing time until diagnosis by 1 μg/L per year ($p = 0.045$) and were not significantly dependent on gender or age. For controls, females were found to have higher s-Tg values than men by 4 μg/L ($p = 0.048$), but this value was not significantly age or time dependent.

The results of measurements divided into 5-year periods before diagnosis for mean s-Tg values, and sensitivity and specificity are presented in Table 4. Sensitivity was around 50 for measurements that were taken up to 15 years prior to diagnosis, but 21 when the interval was over 15 years. Specificity was 89.

Table 5 shows all measurements of cases, including cases with multiple measurements, according to length of time between measurement and diagnosis. Seventeen cases had multiple serum samples, thus adding 27 measurements to the 59 shown in Table 4. Six of those had normal values in all samples. In four cases with multiple samples, measurements have changed from < 30 to ≥ 30 μg/L. These conversions occurred more than 6 years before the diagnosis. Elevated s-Tg values have existed for up to 20 years before carcinoma diagnosis.

DISCUSSION

In Iceland the incidence and mortality rates for thyroid cancer are higher than in most other populations (8, 9). Many patients have high s-Tg values at the time of diagno-

Table 4

Cases, mean s-Tg values, sensitivity and specificity of s-Tg positive cases and controls according to time before cancer diagnosis

Time up until diagnosis	No. of		Mean s-Tg values		Sensitivity	Specificity
Years	Cases	Controls	Cases	Controls		
0.0–4.9	13	58	110	13	46	86
5.0–9.9	15	54	177	15	67	86
10.0–14.9	17	39	45	17	41	93
15.0–19.9	14	23	24	14	21	89
Total (0.0–19.9)	59	164	88	18	44	89

sis. Thoresen et al. (1) have shown that in Norway this elevation began several years before the diagnosis of thyroid carcinoma. In view of the high incidence in Iceland, and the possibility to collect serum samples from individuals subsequently diagnosed with carcinoma of the thyroid, the present investigation was both important and appropriate.

S-Tg concentrations are of great value in the follow-up after treatment of thyroid cancer (3), but the measurement of s-Tg in the serum of these patients is not without problems, as no serum Tg assay today is completely accurate. This is caused by the relatively common presence of anti-Tg antibodies in serum of patients with raised serum Tg levels (10). In Thoresen et al.'s study (1) an immunoradiometric assay (Sorin, Italy) was used to assay s-Tg. The upper limits of reference values for that assay were 150 μg/L for females and 70 μg/L for males, which is much higher than the reference values of the present Tg assay, where the upper limit for both sexes was 30 μg/L.

Earlier polyclonal radioimmunoassays have been shown to produce somewhat higher values than immunometric assays with monoclonal antibodies (10–12), but the latter method does not provide any substantial advantage in detecting Tg in Tg-Ab-positive sera. As we have, however, eliminated all sera with less than 80% recovery, in our serum samples the effects of Tg antibodies on our results should be negligible.

The simultaneous measurements of TSH and T4 in serum of the carcinoma cases and controls show that there is no significant difference between cases and controls with respect to these hormones. Furthermore, the s-Tg values in our population should not be affected appreciably by hyperfunctioning, autoimmune thyroid glands, which generally elevate s-Tg values (13).

This study has shown that up to 20 years can elapse from the time thyroglobulin values became elevated to the time of diagnosis of the clinical case. Assuming that this elevation of thyroglubulin values is caused by a carcinoma or a precancerous condition in the gland, this indicates that the latency period for thyroid carcinomas is anywhere from 6 to 20 years, or more. Even though other diseases of the thyroid gland can cause thyroglubulin elevation, our data indicate that the elevation is a predictor of carcinoma. The odds ratio in our study shows that persons with increased s-Tg values are seven times more likely to become cases than those with levels within the reference limits.

We found elevated thyroglobulin values in 44% of cases, which is considerably higher than the proportion found in Norway (1). In our study the interval between measurement and clinical diagnosis was up to 15 years and therefore our value for the frequency of raised levels may by underestimated. Thyroglobulin has not been used as a screening test in thyroid cancer. The main reason that it cannot be advocated as such is its lack of sensitivity. About 50% of newly diagnosed thyroid cancer patients do not show elevated thyroglobulin values at the time of diagnosis. The specificity of 89 shown in Table 4, could be increased because the false-positive values can be further investigated by blood tests, ultrasound of the thyroid gland and fine-needle aspiration, to differentiate between either benign or malignant causes of the s-Tg elevation.

In our study 18 out of 164 controls (11%) had elevated s-Tg values, but this does not diminish its usefulness in the clinical follow-up of thyroid carcinoma patients. In patients with increased values at the time of diagnosis, increased values after treatment indicate thyroglobulin-producing tissue requiring further treatment. The 18 controls with elevated thyroglobulin values will be followed to determine whether any of them will develop thyroid carcinoma. Another unanswered question is why only 44% of our cases had elevated values. It is hoped that further research will clarify these two questions.

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