

NOTE

Measuring thyroglobulin autoantibodies by sensitive assay is important for assessing the presence of thyroid autoimmunity in areas with high iodine intake

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Abstract. There is some debate over the clinical utility of measuring serum TgAb to assess the presence of thyroid autoimmunity. To clarify the relationship between TgAb levels and thyroid autoimmunity, a histological examination of thyroid tissue was carried out on unselected living individuals with detectable serum TgAb. 146 patients with a pathological diagnosis of follicular adenoma were selected as subjects. Focal lymphocytic infiltration (FLI) was defined as lymphocytic aggregates of more than 200 in number. A thyroid gland in which 0-1 FLI was observed in a few visual fields of low magnification (20×4) in thyroid tissue adjacent to a tumor was judged to be normal and a thyroid gland in which 2 or more FLI were observed was diagnosed as focal lymphocytic thyroiditis (FLT). Serum levels of TgAb and TPOAb were measured by radioimmunoassay. Out of the 146 patients, 18 had detectable serum TgAb and 16 had detectable serum TPOAb. All but one (i.e. 94%) of the 18 TgAb positive patients had FLT and 14 out of the 16 TPOAb positive patients had FLT. The sensitivity (17/32; 53.1%) and specificity (113/114; 99.1%) of TgAb for detecting FLT were higher than those (14/32; 43.7% and 112/114; 98.2%) of TPOAb, but the differences were not significant. In 9 patients who were TgAb positive (but TPOAb negative), 8 (88.9%) had FLT. These results throw doubt on the Laboratory medicine practice guidelines published in Thyroid 2003, in which measuring TgAb is not usually necessary for detecting autoimmune thyroid disease. At least measuring TgAb by sensitive assay is useful for assessing the presence of thyroid autoimmunity in Japan, an area with high iodine intakes.

Key words: TgAb, TPOAb, Focal lymphocytic thyroiditis, Hashimoto's disease, Thyroid histology

AUTOANTIBODIES to thyroglobulin (TgAb) and/or to thyroid peroxidase (TPOAb) are hallmarks of thyroid autoimmunity, but there is some debate over the clinical value of measuring TgAb to assess the presence of thyroid autoimmunity [1, 2, 3]. At least some guidelines for laboratory medicine practice [1] suggest that TgAb measurements are not useful for diagnosing autoimmune thyroid disease. To clarify the relationships among TgAb, TPOAb, and thyroid autoimmunity, the relationship between those antibodies and histological findings of thyroid were investigated using thyroid glands from unselected postmortems [4] or as-

piration or needle biopsy specimens of thyroid glands of patients with enlarged thyroid gland [5, 6]. The ideal approach to clarify this issue is considered to be to analyze the relationships among TgAb, TPOAb, and histological findings of thyroid in unselected living subjects, regardless of whether or not they have Hashimoto's disease. We describe a study in which thyroid tissue adjacent to a follicular adenoma was examined histologically in 146 patients and the results are compared with their serum TgAb and TPOAb levels.

Materials & Methods

Subjects

Out of 454 patients with a benign thyroid tumor operated on at Ito Hospital between 1995 and 1999, 152 patients with a pathological diagnosis of follicular adenoma were selected and the adjacent thyroid tis-

sues studied. Patients with an adenomatous nodule(s), functioning adenoma, and follicular adenoma complicated with adenomatous goiter, thyroid carcinoma, or Graves' disease were excluded. The size of the adjacent tissue was measured, and in 146 patients (male 29, female 117, age 43.3 ± 13.4 ; mean \pm SD y/o) both the length and width of adjacent thyroid tissue in histological section were more than 2mm and these 146 were selected for further study. TgAb, TPOAb as well as free T4 and TSH concentrations were measured in serum samples taken several days prior to surgery.

Histological observations

Thyroid tissue (adjacent to each tumor) was fixed with 10% buffered formalin (pH 7.2), and histological sections stained with hematoxylin and eosin. Focal lymphocytic infiltration (FLI) was defined as more than 200 lymphocytic aggregates. A thyroid gland in which 0-1 FLI was observed in a few visual fields of low magnification (20×4) of thyroid tissue adjacent to a tumor was judged to be normal and a thyroid gland in which 2 or more FLI were observed was diagnosed as focal lymphocytic thyroiditis (FLT).

Measuring antithyroid antibodies, and FT4 and TSH

Serum levels of TgAb and TPOAb were measured by radioimmunoassay [7] using kits provided by Cosmic Corp. (Tokyo, Japan) in accordance with the kit instructions. The minimum detectable level of both TgAb and TPOAb was 0.3 U/mL.

Serum free T4 and TSH concentrations were determined by chemiluminescent enzyme immunoassay using commercially available kits (lumipulse FT4 and lumipulse TSH, Fuji Rebio Inc, Tokyo, Japan).

Statistical analysis

For statistical assessment, Student's t test and chi-square test were used. A probability value of less than 0.05 was considered significant.

Results

Overall, FLT was found in 32/146 (21.9%) of the patients (30/117 (25.6%) for females and 2/29 (6.9%) for males). Histological examination of the entire area of the available thyroid tissue was carried out including tissue close to and distant from the tumour. The sizes of the adenomas of patients with FLT (36.5 ± 27.3 g; mean \pm SD) did not differ from those without

FLT (37.0 ± 27.4 g).

Out of the 146 patients, 18 (12.3%) had detectable serum TgAb and 16 (11%) had detectable serum TPOAb (Table 1). Both antibodies were detectable in 9 (6.2%). All but one (i.e. 94%) of the 18 TgAb positive patients had FLT, and 14 out of 16 TPOAb positive patients had FLT. In 9 patients who were TgAb positive (but TPOAb negative), 8(88.9%) had FLT. FLT was present in 22/25 (88.0%) of patients who had one or both antibodies and in 10/121 (8.3%) patients who did not have either TgAb or TPOAb. The sensitivity (17/32; 53.1%) and specificity (113/114; 99.1%) of TgAb for detecting FLT were higher than those (14/32; 43.7% and 112/114; 98.2%) of TPOAb but the differences were not significant.

Two patients had slightly elevated (4.1 and 4.8 mU/L) serum TSH levels (normal range 0.3-4.0 mU/L) but neither showed FLT or detectable TgAb or TPOAb. Also there was no significant difference in TSH concentrations between patients with FLT (1.61 ± 0.73 mU/L; mean \pm SD) and without (1.43 ± 0.94 mU/L). In addition TSH concentrations were not significantly different in patients with TgAb and/or TPOAb (1.47 ± 0.75 mU/L) and without (1.46 ± 0.92 mU/L).

Discussion

Our studies indicate that in the group of patients we investigated, the presence of TgAb was a particularly good indicator of the existence of FLT with only one TgAb positive patient (5.6%) showing no FLT. The sensitivity and specificity of TgAb for detecting FLT were higher than those of TPOAb, although the differences were not significant.

One of the limitations of our study is that we have examined thyroid tissue adjacent to a tumour and the tumour itself might influence the results. Fiore *et al.* reported that the frequency and severity of lymphocytic infiltration is significantly higher in papillary thyroid cancer than benign thyroid nodular disease [8]. But, only patients with a pathological diagnosis of follicular adenoma were selected in this study. There was no relationship between tumour size and the presence of lymphocytic infiltration in the adjacent tissue. Also, lymphocytic infiltration was observed in tissue close to and distant from the tumour. In addition, the prevalence of TgAb and TPOAb in the group of patients we studied was similar to the prevalence observed in the general population with the same assay

Table 1 Relationship between TgAb, TPOAb and histological findings for thyroid tissue adjacent to tumor

	NUMBER OF PATIENTS	NORMAL HISTOLOGY		FOCAL LYMPHOCYtic THYROIDITIS (FLT)	
TgAb(+)	18	1	5.6%	17	94.4%
TPOAb(+)	16	2	12.5	14	87.5
TgAb(+) & TPOAb(-)	9	1	11.1	8	88.9
TgAb(-) & TPOAb(+)	7	2	28.6	5	71.4
TgAb(+) & TPOAb(+)	9	0	0.0	9	100.0
TgAb(+) or TPOAb(+)	25	3	12.0	22	88.0
TgAb(-)	128	113	88.3	15	11.7
TPOAb(-)	130	112	86.2	18	13.8
TgAb(-) & TPOAb(-)	121	111	91.7	10	8.3
Total	146	114		32	

methods [9], and the prevalence of FLT in the group of patients is also similar to the prevalence of focal thyroiditis observed in post-mortem tissue [10, 11]. The presence of serum TgAb and/or TPOAb has been found to be associated with FLT in post-mortem tissue specimens [4] and biopsy samples from patients with enlarged thyroid glands [5]. The current study however has an advantage over these earlier reports as there was no age based bias (as in the case of post-mortem samples) or selection on the basis of large thyroid glands.

According to the laboratory medicine practice guidelines published in Thyroid, TPOAb is the most sensitive test for detecting autoimmune thyroid disease, and it is not usually necessary or cost-effective to order both TPOAb and TgAb in iodide-sufficient areas [1]. However, the relationship between TgAb and histological findings of thyroid was not analyzed in the guideline. In this study, TgAb was thought to be the same or more closely related to the histological findings of autoimmunity than TPOAb and at least 8 patients with positive TgAb and negative TPOAb had histological findings of autoimmunity. While the analysis was performed using a different approach, with biopsy samples obtained from patients with goiter [5], and post-mortem thyroid tissue [4], it has been reported that a histological diagnosis of Hashimoto's thyroiditis can most precisely be predicted by radioimmunoassay for TgAb in comparison to TPOAb, and thyroglobulin antibody and microsomal antibody measured by hemmagutination technique in Japan. Baker *et al.* reported that a cytologic diagnosis of Hashimoto's thyroiditis correlated better with antimicrosomal antibodies than with antithyroglobulin an-

tibody titers, but those antibodies were measured by a hemagglutination technique, which is less sensitive than TgAb and TPOAb [6]. Regrettably, there are no reports from Europe and the United States on analyses of the relationship between TgAb, TPOAb measured by sensitive assay and histological findings. In 2 recent reports from Europe on the association between thyroid autoimmunity and various thyroid tumors, the difference between the significance of TgAb and TPOAb was not analyzed in relationship to histological findings [8, 12].

One of the reasons why TPOAb measurements are to be preferred over TgAb measurements for detecting autoimmune thyroid disease, as written in the guideline in the USA, is that TPOAb-negative patients with detectable TgAb rarely display thyroid dysfunction [2]. However, Konno [13] has reported a significant correlation between TgAb levels (with or without detectable TPOAb) and TSH levels in 2,647 ostensibly healthy Japanese subjects. We also reported that the incidence of hypothyroidism in untreated patients with Hashimoto's disease who were only TgAb positive (66/414 cases; 15.9%) was higher than in patients who were only TPOAb positive (4/33 cases; 12.1%), although the difference was not significant [14]. Furthermore, Kasagi *et al.* reported that the prevalence of TgAb was higher than TPOAb in subjects with subclinical hypothyroidism [15].

Certainly, TPOAb is more relevant than TgAb among those with elevated TSH in reports from Europe and the United States [2, 16, 17, 18, 19]. The prevalence of TgAb is higher than that of TPOAb in patients with Hashimoto's disease in reports from Japan [4, 5], but not in the report from Europe [7].

The significance of measuring TgAb for detecting autoimmune thyroid disease is thought to be different in Japan compared to Europe and the United States. Li *et al.* reported that constant exposure to an excessive iodine intake increased the incidence of positive TgAb, and only TgAb-positive subjects from areas with relatively high iodine intakes were more likely to develop hypothyroidism than only TPOAb-positive subjects [20]. The average dietary iodine intake due to the ingestion of seaweeds is 1.2 mg/day in Japan [21]. The difference in iodine intake between Japan and Europe, United States may cause the difference in the significance of TgAb for detecting autoimmune thyroid disease [22].

Overall, therefore, our studies indicate that the presence of TgAb as measured by sensitive direct radio-

immunoassay is almost always associated with FLT and TgAb was the same or more closely related to the histological findings of autoimmunity than TPOAb. These results throw doubt on the Laboratory medicine practice guidelines published in *Thyroid 2003*, in which measuring TgAb is not usually necessary for detecting autoimmune thyroid disease. At least measuring TgAb by sensitive assay is useful for assessing the presence of thyroid autoimmunity in Japan, an area with high iodine intakes.

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