

Brief Report

Autoantibodies Highly Increased in Patients with Thyroid Dysfunction

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To evaluate the significance of antithyroid antibody levels, five hundred and twenty-six patients with thyroid diseases and 292 health subjects from Yuci District, Shanxi Province, China, were studied. Serum levels were determined for thyroid hormone receptor antibody (TRAb), microsomal antibody (TMAb) and thyroglobulin antibody (TGAb). Among patients, the percentages for nodular goiter and thyroid adenoma, Graves' disease, and Hashimoto's thyroiditis are 44.1%, 19.6% and 17.7%, respectively. The ratios of female to male were 2.0 to 15.6. Antibody-positive patients for TMAb, TGAb and TRAb were detectable as 94.6%, 76.3% and 20.4% for Hashimoto's thyroiditis, and 40.0%, 30.0% and 90.3% for Graves's disease. In conclusion, the high levels of the TRAb in Graves' disease, and those of the TGAb/TMAb in Hashimoto's thyroiditis and idiopathic hypothyroidism are meaningful for characterizing the epidemiological basis of the diseases and for using as prognostic indicators for the relapse in individual patients. *Cellular & Molecular Immunology*. 2007;4(3):233-236.

Key Words: thyroid disease, TGAb, TMAb, TRAb

Introduction

The thyroid is a small gland to control the body's metabolism by producing hormones, T4 and T3, which tell the body's cells how much energy to use. There are several different causes of thyroid disease. The term autoimmune thyroid disease encompasses a diverse range of clinical entities. Classically, autoimmune diseases are characterized by the activity of autoreactive lymphocytes, which cause tissue or organ damage through the formation of antibodies that react against host tissues, or effector T cells, which are specific for endogenous self-peptides (1, 2). Autoimmunity to thyroid antigens leads to two distinct pathogenic processes with opposing clinical outcomes: hypothyroidism in Hashimoto's thyroiditis and hyperthyroidism in Graves' disease (3-5). The high frequency of these diseases and easy accessibility of the thyroid gland have allowed the identification of key pathogenic mechanisms in organ-specific autoimmune diseases (6, 7). In early investigations, antibody- and T-cell-mediated death mechanisms were proposed as being responsible for autoimmune thyrocyte depletion (7).

Autoimmune thyroid disease is the predominant form of thyroid dysfunction in the developed world. There is a vigorous debate about the management of the increasingly commonly recognized subclinical forms of thyroid dysfunction despite recent recommendations (8). There is few data supporting associations of subclinical thyroid disease with symptoms or adverse clinical outcomes or benefits of treatment. The consequences of subclinical thyroid disease (serum thyroid stimulating hormone (TSH) 0.1-0.45 mIU/L) are minimal. There is insufficient evidence to support population-based screening (9). Meanwhile, thyroid hormone regulation of postembryonic development occurs in all vertebrates, and thyroid hormone deficiency has profound effects during human gestation, leading to cretinism, a disease characterized by short stature and mental retardation (10). Thyroid hormone receptor acts as a hormone-dependent transcriptional transactivator and as a transcriptional repressor in the absence of thyroid hormone. Specifically, thyroid hormone receptor can repress retinoic acid-induced gene expression through interactions with retinoic acid receptor. Thyroid hormone receptor antibodies (TRAbs) have been identified as the antibodies responsible for the are pathophysiological and clinical indicators in autoimmune thyroid diseases, not only in Graves' disease (11) while blocking TRAb have been implicated as the cause of hypothyroidism in some patients with chronic lymphocytic thyroiditis (CLT). Although thyroglobulin antibodies (TGABs) are recognized markers of autoimmune thyroid disease, they lack a defined biological action. Thyroid microsomal antibodies (TMABs) are found in the serum of almost all patients with Hashimoto's thyroiditis, in more than 70% of those with Grave's disease, and, to a variable degree, in patients with

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Table 1. Clinical characters of all patients

Diseases	Total (%)	Male	Female	F:M
Nodular goiter and thyroid adenoma	232 (44.1%)	14	218	15.6
Graves' disease	103 (19.6%)	17	86	5.1
Hashimoto's thyroiditis	93 (17.7%)	12	81	6.8
Idiopathic hypothyroidism	39 (7.4%)	13	26	2.0
Subacute thyroiditis	37 (7.0%)	9	28	3.1
Thyroid cancer	22 (4.2%)	4	18	4.5
Total	526	69	457	6.7

non-thyroid autoimmune diseases and some normal subjects. Their titres do not correlate with thyroid functional status and do not necessarily fall with therapy. Thus, TMABs are also established markers of autoimmune thyroid disease, and again, their pathogenic role is not clearly defined.

Herein, we set up reference distributions and decision values for thyroid antibodies against TGAb, TMAB, and TRAb to distinguish the clinical characters of thyroid diseases of local residents.

Material and Methods

Patients

All patients attending the First People's Hospital of Yuci District (Jinzhong City, Shanxi, China) between January 2002 and December 2006 were investigated according to provincial guidelines. A total of 526 patients (median age 41.3, range 13-75) with thyroid disease were investigated, and the overall female-to-male ratio was 6.7 (457:69). Also 292 normal people were recruited as control including 71 males and 221 females (median age 43.5, range 17-72). These individuals did not smoke and had no goiter or no personal or family history of thyroid disease, and thyroid autoantibodies were negative.

Thyroid antibodies assay

Blood samples were collected in anticoagulant-free tubes and centrifuged at 1,000 g for 10 min at 4°C. Sera were decanted for storage at -20°C until assay. The principle of the test was to identify TGAb and TMAB by using indirect particle agglutination. Specimens of patient serum were tested using commercially made kits manufactured by Tianjin Union Medical Tech., China. In brief, sera were added to increasingly diluted serum samples to 10 IU wells on a microtitre plate. Sensitized particles were added to each well, and unsensitized particles were added to an additional serum sample of the lowest dilution. The plates were incubated overnight at room temperature. Agglutination patterns of unsensitized particles and negative titres formed button-shaped patterns as the particles concentrated in the centre of the well. Positive titres showed a film of agglutinated particles substantially larger than the button in the negative wells. A test sample which showed a negative result with unsensitized particles and showed agglutination with sensitized particles at a dilution

more than 1:100 was interpreted as positive and reported as a titre (the highest dilution to give a positive well). The titre > 1:100 for TGAb or > 1:400 for TMAB were considered positive.

In the TRAb assay, TRAb ELISA kits (Tianjin Union Medical Tech., China) were applied. Briefly, 100 µl of test sera were added to duplicate receptor-coated wells, followed by incubation at room temperature for 2 h. Sera were then removed (without washing at this stage), and 100 µl (5 ng) of biotinylated bovine TSH in assay buffer added. After further incubation for 15 min at room temperature, excess TSH-bi was removed (without washing at this stage), 100 µl of streptavidin-peroxidase conjugate was added, and incubation was continued for 20 min. The wells were then washed once with assay buffer and once with distilled water. The peroxidase substrate tetramethylbenzidine (100 µl) was added to the wells, followed by, after 20 min, the addition of 2 mol/L H₂SO₄ and measurement of absorbance at 450 nm.

Statistical analysis

Quantitative variables were analyzed using nonparametric tests. The Mann-Whitney test was used to compare the variables in the different groups.

Results

The clinical characters of patients

Our hospital mainly served for the health maintaining of local residents. But from this community restricted data, it was found that large percent of patients were suffered from nodular goiter and thyroid adenoma (44.1%), Graves' disease (19.6%) and Hashimoto's thyroiditis (17.7%). On the other hand, we did find that thyroid disease affects more women than man. Our study showed significant sex-dependence in terms of the percentage of thyroid nodular disease. For different group, the female to male ratio was 2.0 to 15.6 (Table 1).

Thyroid antibody detection

On reviewing data collected for this study since 2002, the related diagnostic specificities were presented in Table 2. There was no significant difference in age or sex between the thyroid antibody positive and negative groups within the whole study population. Of the normal 292 people, only 3

Table 2. The diagnostic specificity of patients at the time of diagnosis

Diseases	TGAb (%)	TMAb (%)	TRAb (U/L)
Nodular goiter and thyroid adenoma	M:3.87 (0.39-30.11) F: 3.98 (0.57-29.35)	M:6.71 (0.28-40.12) F: 6.38 (0.54-45.25)	M: 0.98 (0.02-15.98) F: 0.87 (0.01-16.84)
Graves' disease	M: 15.69* (1.27-40.89) F: 16.32* (0.75-57.61)	M: 19.28* (0.99-45.26) F: 19.89* (0.66-59.34)	M: 37.65* (3.12-186.69) F: 40.26* (2.98-209.35)
Hashimoto's thyroiditis	M: 28.63* (5.13-61.24) F: 36.14* (8.82-67.99)	M: 32.4* (6.12-70.32) F: 42.02* (9.37-75.45)	M: 1.32 (0.06-50.23) F: 2.05 (0.86-95.36)
Idiopathic hypothyroidism	M: 24.06* (4.66-62.61) F: 19.86* (4.97-70.65)	M: 35.66* (7.98-74.30) F: 33.26* (8.32-76.66)	M: 4.52* (0.02-102.26) F: 3.98* (0.05-98.26)
Subacute thyroiditis	M: 13.86* (2.37-28.96) F: 12.14* (1.89-46.95)	M: 21.32* (5.39-49.75) F: 20.84* (5.32-50.84)	M: 2.89* (0.14-58.44) F: 2.36* (0.03-46.32)
Thyroid cancer	M: 11.75* (1.47-40.62) F: 10.69* (0.26-28.07)	M: 13.26* (3.25-42.81) F: 11.97* (0.66-35.32)	M: 1.78 (0.02-15.26) F: 1.85 (0.33-26.39)
Normal	M: 3.88 (0.41-28.39) F: 4.12 (0.59-31.17)	M: 6.77 (0.35-38.65) F: 6.81 (0.46-41.02)	M: 0.87 (0.01-13.32) F: 0.91 (0.01-16.25)

The data are presented as mean with ranges in brackets. *, $p < 0.05$, compared with normal.

(for TGAb and TMAb) or 4 (for TRAb) cases showed thyroid antibody positive. At diagnosis of 93 patients with Hashimoto's thyroiditis, 88 (94.6%) were proved to be TMAb positive, 71 (76.3%) patients were TGAb positive, but only 19 (20.4%) were TRAb positive. Among 103 Graves' patients, there were 93 (90.3%) cases identified TRAb positive, but only 30%, 40% patients shown TGAb, TMAb positive, respectively. But for the patients with nodular goiter, subacute thyroiditis and thyroid cancer, thyroid antibody diagnosis proved less percentage of positive, which render a drive force for other laboratory detection (Figure 1).

Discussion

Autoimmune thyroid disease is the archetype for organ-specific autoimmune disorders. Progress in treating these disorders lies in the improvements of our understanding of the predisposing factors, the mechanisms responsible for the progression of disease, and the interaction between thyroid antigens and the immune system at the level of T cell and antibody (12). This interest is sparked by the need to understand these unusual antibodies which have such obviously potent pathological effects despite their low serum concentration relative to other autoantibodies (13). It was shown by population based screening that the prevalence of thyroid diseases are greater in females than male, and patients with nodular goiter/thyroid adenoma were more frequent than other thyroid diseases. Our findings provide improved means for characterizing the epidemiological basis of this pathogenic response by the means of measuring TGAb, TMAb, and TRAb.

In common with other autoimmune diseases, genetic, environmental and endogenous factors are required in an appropriate combination to initiate thyroid autoimmunity (14). The aetiology and mechanics of the autoimmune cellular and antibody responses involves a combination of human leucocyte antigen (HLA) linkage, genetics and environmental factors, including smoking (15), stress and iodine intake, to

determine the initial and subsequent stages of the development of autoimmune thyroid disease (4). Both Graves' disease and Hashimoto's thyroiditis are well-characterized diseases that are often diagnosed on the basis of clinical impression. Laboratory and pathological findings often provide important confirmatory information. Graves' disease is thought to represent an autoimmune process of the thyroid gland in which stimulatory autoantibodies bind to the TSH receptor and activate gland function leading to hyperthyroidism, often accompanied by thyromegaly. The disease is accompanied by a number of symptoms directly referable to thyroid hormone excess (16). In accordance with other study, we found that TRAb assay had the highest diagnostic power to differentiate Graves' disease from toxic multinodular goiter (17), when clinical features were not conclusive since it was detected, more than 90% patients with Graves' disease were proved TRAb positive. TGAb and TMAb are particularly useful as they may predict Hashimoto's thyroiditis and idiopathic hypothyroidism.

Factors such as genetic background, age, and gender partially account for the development of thyroid diseases. As with other autoimmune diseases, an environmental factor also has long been suspected in the etiology of those diseases (16). As we indicated, there is 6.7 fold for female patients compared to male ones. It was demonstrated that sex hormones play a role in the genesis of autoimmunity (18), and X chromosome inactivation (XCI) and resultant tissue chimerism could offer an explanation for the female predisposition to thyroid autoimmunity, too (19). Meanwhile, we did find that high frequency of nodular goiter and thyroid adenoma was diagnosed, and this phenomenon could be rendered as an indicator for local public health improvement (20) and allowing for recommendations on salt-iodine enrichment in the future (21), because Jinzhong area is one of the prevalence sites for goiter and iodine deficiency (22).

Our report also has several limitations. First, as a community hospital, our patients are mainly local residents, even much from sub-urban or rural areas. It will be helpful to assess whether a relationship exists between thyroid anti-

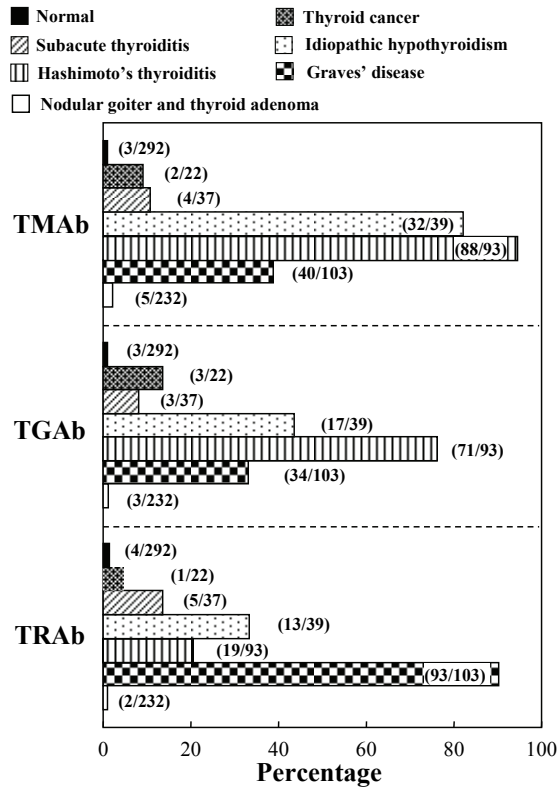


Figure 1. The percentage of proved positive thyroid antibodies of whole study population. From a whole study group of healthy blood donors (n = 292) and thyroid patients (n = 526), microsomal antibody (TMAb), thyroglobulin antibody (TGAb) and thyroid hormone receptor antibody (TRAb) concentrations were measured and positive cases were identified. The number in the brackets was “number of positive individual/whole study group”.

bodies and increased aggressiveness of thyroid disease, but we failed to trace the patients after they checked out, partly because of difficulties of contacting and financial burden, which are often accompanied with health issue of common Chinese people (<http://www.moh.gov.cn/newshtml/19256.htm>). Secondly, current preoperative methods of determining thyroid diseases and assessing their prognosis are unsatisfactory. It was necessary to apply advanced technology to measure these subtypes for thyroid auto-antibody with potential clinical implications, for example, in predicting responsiveness to treatment in untreated patients with thyroid disease (23). Thirdly, our recommendations are based on the existing evidence and clinical experience, but they are limited by the paucity of definitive data. Well-conceived and executed intervention trials are needed to bring definitive data to light on these questions (9). Until such data are available, clinical judgment and patients' preferences remain paramount.

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