MICROSOMAL AND THYROGLOBULIN ANTIBODIES IN THYROID DISORDERS

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ABSTRACT

Eighty patients with various thyroid disorders and 20 controls were investigated to determine antibodies against thyroglobulin and microsomal antigens by haemagglutination and immunofluorescent techniques. They were also tested for immunoglobulin profile by radial immunodiffusion methods. Patients with Hashimoto's disease, idiopathic hypothyroidism, and Grave's disease showed significant elevations of IgG. None of the patients with toxic nodular goitre or thyroid adenoma showed any thyroid antibodies while 69.2% patients with Grave's disease, 16% with euthyroid goitre, 58.3% with hypothyroidism and all the patients with Hashimoto's disease had microsomal antibodies by immunofluorescent test. Similarly, all the cases of Hashimoto's disease, 41.6% of those with hypothyroidism, 30% of Grave's disease patients and none of the patients with toxic nodule or thyroid adenoma had thyroglobulin antibodies by haemagglutination test. Evaluation for auto-antibodies may aid the clinician in the overall assessment of various thyroid disorders.

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KEY WORDS : Autoantibodies; Microsomal antigen; Thyroglobulin.

Introduction

Autoimmune responses to several tissue-specific antigens of thyroid gland are involved in the pathogenesis of chronic thyroiditis and many other thyroid disorders [1]. Of these, antibodies to thyroglobulin (TG) and to microsomal antigen (now called thyroid peroxidase or TPO) are of particular diagnostic importance [2,3]. The present study describes the prevalence of these auto-antibodies in diverse thyroid disorders.

Material and Methods

The study was carried out on 80 patients with thyroid disorders attending the thyroid clinic of a referral hospital and 20 normal healthy subjects. Thyroglobulin antibodies (TGA) were detected by haemoagglutination test (HA) using Thymune-T (WELCOME) kit. The test utilizes turkey red cells coated with human thyroglobulin which agglutinate in

the presence of specific autoantibodies in U-well microtitre plates.

The technique of immunofluorescence (IF) was used for detecting anti-microsomal and thyroglobulin antibodies. The fluorakit supplied by M/S Clinical Sciences Inc was used in the present study. In this test, monkey thyroid tissue was used as a substrate. Cryostat section of the tissue was overlaid with a prediluted control serum or diluted patients serum and incubated in a moist chamber at room temperature. The unbound sera was washed from the slide with phosphate buffered saline (PBS). Fluorescein isothiocyanate (FITC) conjugated rabbit anti-human globulin at optimum dilution and containing Evans blue as a counter-stain was overlaid on the tissue and the incubation step repeated. Unbound conjugate and excess counter-stain was washed from the slide with PBS. Buffered mounting medium was applied and the slide cover slipped. The

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reactions were observed with a fluorescent microscope. In a positive serum, either anti-microsomal antibody stained the cytoplasm of thyroid epithelial cells lining the follicle or thyroglobulin antibody stained as a mesh-work pattern which corresponds with the typical distribution of thyroglobulin in the lumen of thyroid follicles. The test was considered weakly positive if fluorescence was observed using a serum dilution of 1:20 and strongly positive in a serum dilution of 1:40 or above.

Immunoglobulins IgA, IgG, and IgM in the serum were estimated by single radial diffusion method using Tripartigen plates obtained from Hoechst Lab. The concentrations of immunoglobulins were expressed as mg/dL.

Observations

Eighty patients with various thyroid disorders and 20 normal healthy controls were studied. Patients included those with primary thyrotoxicosis (13), Hashimoto's goitre (3), primary hypothyroidism (24), nontoxic diffuse or nodular goitres (25), toxic nodular goitre (6), thyroid adenoma (5), euthyroid or mildly thyrotoxic goitre due to hormone defects (4). The prevalence of auto-antibodies in various thyroid disorders is shown in Table 1.

Primary Thyrotoxicosis : Of the 13 patients, nine (69.2%) showed presence of microsomal antibodies. Thyroglobulin antibodies as assessed by IF were detected in 3 (23%) and by HA in 4 (30%).

Toxic Nodular Goitre : None of the 6 patients with secondary thyrotoxicosis had any demonstrable antibodies either to microsomal antigen or to thyroglobulin.

Euthyroid Goitre : Only 4 (16%) of the 25 cases with simple goitre showed presence of microsomal antibodies only at low dilution (1/20). Thyroglobulin antibodies were absent in all cases. Patients with thyroid adenoma and those due to dysmorphogenesis did not show antibodies either to microsomal antigens or to thyroglobulin, both by IF and HA.

Primary Hypothyroidism : Twenty four patients with primary hypothyroidism were studied. Fourteen patients (58.3%) showed strong positivity (serum dilution of 1:40 or above) for microsomal antibodies. Thyroglobulin antibodies in significant titres (> 1/40) were detected in 10 patients (41.6%) both by IF & HA.

Hashimoto's Thyroiditis : Both microsomal and thyroglobulin antibodies were detected in the 3 cases of Hashimoto's thyroiditis. These were histologically proved and had diffuse lymphocytic thyroiditis and markedly raised (> 1/640) titres of thyroglobulin

TABLE 1
Distribution of autoantibodies in patients with thyroid disorders (n=80)

Thyroid Disorder (No. of patients)	IF-TPO (%)	IF-TG (%)	HA-TG (%)
Primary Thyrotoxicosis(13)	9 (69.2)	3 (23)	4(30)
Toxic Nodular Goitre(6)	0	0	0
Euthyroid Goitre(25)	4 (16)	0	0
Thyroid Adenoma(5)	0	0	0
Dyshormogenesis(4)	0	0	0
Primary hypothyroidism(24)	14 (58.3)	10 (41.6)	10 (41.6)
Hashimoto's disease(3)	3 (100)	3 (100)	3 (100)
Controls(20)	0	0	0

IF-TPO = Antimicrosomal antibodies detected by immunofluorescence

IF-TG = Thyroglobulin antibodies detected by immunofluorescence

HA-TG = Thyroglobulin antibodies detected by haemagglutination

TABLE 2
Immunoglobulin levels in thyroid disorders (in mg/dL $\pm$ SD)

	IgG	IgA	IgM
Controls (20)	1420 $\pm$ 240	260 $\pm$ 108	120 $\pm$ 40
Primary thyrotoxicosis (13)	2275 $\pm$ 480*	242.5 $\pm$ 85.3	190 $\pm$ 50
Solitary and multinodular goitre (6)	1720 $\pm$ 300	252 $\pm$ 150	130 $\pm$ 60
Hashimotos thyroiditis and primary hypothyroidism (27)	2460 $\pm$ 620*	295 $\pm$ 102	214.7 $\pm$ 93.4*

(Asterisk indicates values significantly different from corresponding values in control group, $p < 0.01$)

detected by HA. Haemagglutination is a more sensitive test compared to IF for estimation of thyroglobulin antibodies but difference was not statistically significant ($p > 0.05$).

Controls : None of the 20 healthy controls had either microsomal or thyroglobulin antibodies in their sera.

Immunoglobulins : In patients with hypothyroidism and Hashimoto's thyroiditis, IgG and IgM levels showed significant elevation compared to the controls ($p < 0.01$, Table 2). A significant rise in IgG alone was seen in patients with Grave's disease ($p < 0.01$). Immunoglobulin levels did not show any difference compared to controls in other thyroid disorders ($p > 0.05$).

Discussion

There are several thyroid antigens which can generate autoantibodies, namely thyroglobulin, thyroid peroxidase (or the microsomal antigen), a 'second colloid' antigen, TSH-receptors and the 54-, 64-, and 114-kD proteins [4]. At present, the first two are easily evaluated by the HA and IF techniques but the others are still confined to research programmes and cannot be applied routinely. Since the antibodies vary independently of each other in patients serum, it is usually necessary to combine at least two of the tests, one for thyroglobulin and one for microsomal antigen (thyroid peroxidase).

Our results indicate that in primary thyrotoxicosis (Grave's disease) approximately 70% of patients have antimicrosomal antibodies (thyroid peroxidase) while thyroglobulin antibodies are present in upto 30%

cases. These results are in conformity with those reported by Bell and Solomon [6] who found 80% positivity of thyroid peroxidase microsomal antibodies in such cases. According to some, strongly positive microsomal antibodies in thyrotoxic patients may signify the risk of subsequent hypothyroidism, whether the patient is treated by drugs or surgery [8]. This was, however, not evaluated in the present study. None of our patients were on anti-thyroid drugs before assessment of autoantibodies

In toxic nodular goitre, in contrast, both microsomal and thyroglobulin antibodies were absent, implying lack of immune involvement in these cases. Antimicrosomal and thyroglobulin antibodies were likewise infrequent in patients with non-toxic goitre and adenomas. Only 4 of 25 cases studied demonstrated weakly positive antibodies. The antibody titres in non-toxic goitres correlate with the extent of lymphocytic thyroiditis. The results reported in the literature are variable ranging from nil to 40% [6,7,9]

Antibodies have been detected in considerable proportion of patients with primary hypothyroidism [2,4]. They indicate an underlying pathological process thought to be primarily cell mediated damage by thyroid antigen sensitive T-lymphocytes. Anti-thyroid antibodies seem to correlate with the histopathological evidence of lymphocytic infiltration [7]. In the present study, 58% of patients demonstrated significant antimicrosomal antibodies and thyroglobulin antibodies were present in over 40% of cases

Thyroid antibodies, against microsomal

antigen and thyroglobulin, were present in 3 cases of Hashimoto's disease studied. Significantly raised titre of antibody to thyroglobulin ($> 1/640$) were regularly seen. These findings are in conformity with those of other workers. Raised titres of this magnitude are very helpful in diagnosis and differentiate this condition from simple colloid goitre in which the test for antibodies are usually negative. Autoantibodies to thyroglobulin have been reported in 3-18% of individuals with no clinical evidence of thyroid disease. Appearance of autoantibodies is seen usually in females 40-60 years of age and probably signifies sub-clinical focal thyroiditis in these cases.

Significantly raised IgG in Grave's disease and both IgG and IgM in primary hypothyroidism and Hashimoto's may be indicative of the immune participation in the thyroid disorders. Simple colloid goitre and cases of toxic goitre, adenomas and dysmorphogenesis did not show any difference in the serum immunoglobulin levels between patients and controls implying absence of involvement of humoral immune mechanism in these cases.

Antibody evaluation forms only part of the clinical and laboratory investigation of pa-

tients with thyroid disease and can only be fully interpreted in this context. Evaluation for autoantibodies may aid the clinician in the overall assessment of various thyroid disorders.

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