THYROID AUTOANTIBODY MEASUREMENT BY ENZYME IMMUNOASSAY

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ABSTRACT

Thyroid antibodies are commonly utilized in the assessment and diagnosis of autoimmune thyroid disorders. We compared the measurements of antithyroglobulin and antithyroidperoxidase antibodies by enzyme immunoassay with that of the conventional agglutination method. This fully automated enzyme immunoassay is more specific and cost effective than the agglutination method. Further this is a very quantitative and rapid method producing results in two hours as compared to at least twenty= four hours required by the conventional method. Antithyroidperoxidase antibodies determined by enzyme immunoassay are more specific and sensitive in the diagnosis of Hashimoto's thyroiditis than the antithyroglobulin antibodies.

KEYWORDS: EIA, antithyroidperoxidase antibodies, antithyroglobulin antibodies.

INTRODUCTION

Autoimmune thyroid diseases constitute a considerable proportion of patients with thyroid disorders in areas of iodine sufficiency. Antithyroidantibodies are therefore quite commonly performed tests. High titres of anti-thyroglobulin autoantibodies [anti-TG ab) and/or anti-thyroid peroxidase autoantibodies [anti-TPO ab) are mainly associated with autoimmune thyroid disorders and low concentrations are found in a significant proportion of the normal population (1, 2).

Anti-TG ab are circulating immunoglobulins directed against different epitopes of the thyroglobulin molecule. Thyroid microsomal antibodies are circulating immunoglobulins directed against a component of the smooth endoplasmic reticulum of thyroid cells which are identical with thyroid peroxidase. Therefore the microsomal antibodies are also termed anti-TPO ab (3, 4).

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Conventionally, antithyroid antibodies have been measured by partial haemagglutination technique (PHA) using tanned sheep red cells sensitized with appropriate antigen, or an agglutination test (AT) based on antigen coated gelatin particles. These are time consuming and express results as the dilution of serum which causes agglutination. These methods give only semiquantitative information on the circulating level of antibodies. More recently enzyme immunoassays (EIA) have been developed which yield rapid quantitative results.

In this study anti-TG ab and anti-TPO ab were measured in sera from patients with different thyroid diseases using a recently introduced ENZYMUN-TEST (Boehringer Mannheim) and were compared with results obtained by AT technique. Normal ranges for a healthy population group were also established.

PATIENTS

67 patients were investigated; Hashimoto's thyroiditis (11), Graves' disease (5), Multinodular Goiter (19), thyroid cancer (16) and non autoimmune benign thyroid disorder (16). All patients were seen at our endocrine outpatient clinic.

The diagnosis of Hashimoto's thyroiditis was confirmed by a clinical diagnosis of hypothyroidism, a gland which appeared lobular and firm on palpation and an elevated antimicrosomal antibody titre of >40[2] by AT. Graves' disease was diagnosed by clinical and biochemical features of thyrotoxicosis with elevated diffuse radioactive iodine [I 131] uptake. A multinodular goiter was diagnosed on the basis of a clinically nodular gland, normal FTC, TSH, I 131 scan showing patchy uptake and negative antibody titre by AT. Thyroid carcinoma was established by fine needle aspiration cytology [FNAC] test and confirmed by the postoperative histological examination of the tissue. Non-autoimmune thyroid disorders include largely those with solitary nodule and a colloid goiter with a benign FNAC or De Quervain's thyroiditis.

The healthy population group consisted of 50 hospital staff with a mean age of 35 years (range 20 to 60) with an equal number of males and females.

METHODS

The principle of ENZYMUN-TEST, by Boehringer Manheim involves a 3 step sandwich assay using streptavidin technology, which measures the autoantibody levels and expresses the concentration in IU/ml. This method is highly specific when compared with other methods (1, 5, 6, 7). No interference was found in sera containing rheumatoid factor, or antibodies against TSH receptor, DNA, acetylcholine receptors or mitochondria. Further, anti-TPO and anti-TG antibodies did not show any mutual interference in this assay system. [Boehringer Mannheim, West Germany, Immunodiagnostics Manual, Cat. No. 1491148 and 1491130]. Thyroid microsomal antibodies and thyroglobulin antibodies were also measured by AT method [Serodia-ATG, Serodia AMC, Fujirebio, Inc. Japan]. Correlation of the antibody levels obtained by the two methods was studied after log transformation of data.

RESULTS

Table 1 shows a comparison of anti-TG ab levels and anti-TPO levels obtained by both the methods for all 67 patients suffering from different disorders. The intra—assay and inter assay precision data obtained by EIA method are quite good as shown in Table 2. The overall frequencies for negative and positive results obtained with the two methods are summarized in Table 1. Positive results were found in 2% of the samples tested for anti-TG ab by AT method and 55% by EIA method suggesting improved sensitivity of EIA. On the other hand 24% of the samples tested for anti-microsomal autoantibody levels by AT method, and 31% samples tested for anti-TPO ab levels with EIA method were positive.

Table 1: Thyroid autoantibody levels in sera from patients with thyroid disorders-comparison of two methods for detection of antithyroglobulin and antithyroid peroxidase autoantibodies.

Results (%)	AT	EIA
Antithyroglobulin autoantibody:		
Negative	66 (98%)	31 (47%)
Positive	1 (2%)	36 (53%)
Normal range	<40*[2]	0-28*
Antithyroid peroxidase autoantibody:		
Negative	51 (76%)	46 (69%)
Positive	16 (24%)	21 (31%)
Normal range	<40*[2]	0-7**
Number of samples	67	67

* titre
** IU / ml

lable 2: Intra-assay and Inter-assay Precision (EIA).

	Anti-TG ab			Anti-TPO ab		
	$\bar{x}$	n	%cv	$\bar{x}$	n	%cv
	IU / ml		IU / ml			
Intra-assay	42.9	10	4.15	5.2	10	4.21
	579	10	2.65	91.3	10	3.49
Inter-assay	46.2	5	7.90	5.04	5	5.16
	592	5	6.22	87.40	5	5.33

The distribution of anti-TG ab and anti-TPO ab levels in serum of patients with different thyroid disorders obtained by the EIA method are shown in Fig 1 and 2 respectively. Patients with Hashimoto's thyroiditis had elevated levels of both anti-TG ab and anti-TPO ab, however the anti-TPO ab levels were far higher than normal controls indicating that anti-TPO has greater discriminatory value. Patients with Graves' disease had anti-TG ab levels ranging widely between 28 and 1000 IU/ml and anti-TPO ab between 8 and 1000 IU/ml. Most patients with multinodular goiter had both antibody levels in the normal ranges with few sporadic cases with mildly elevated levels. Those with thyroid carcinoma had anti-TG ab ranging from the upper limit of normal to mildly elevated values and these overlap with levels seen in Hashimoto's thyroiditis. On the other hand, anti-TPO ab levels in the same patients which varied from normal to mildly elevated values showed no overlap with levels seen in Hashimoto's thyroiditis. Patients with benign non-autoimmune disease had a wide scatter for both anti-TPO and anti-TG levels.

Fig. 1

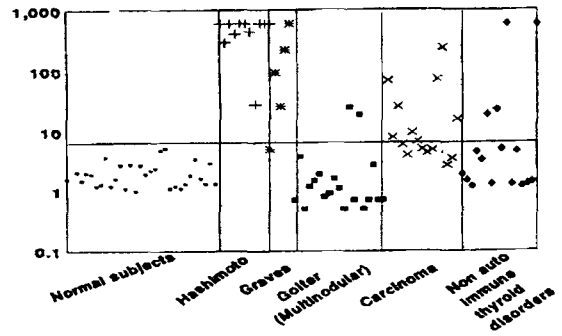

Fig-2

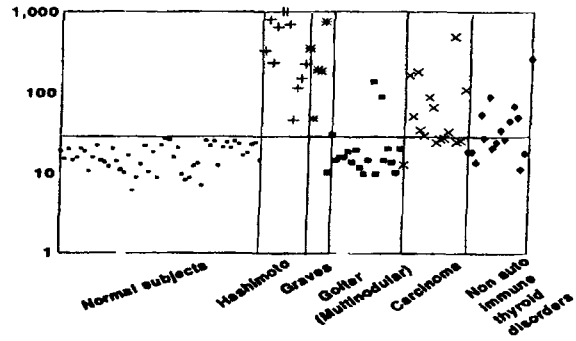

Fig. 1: Distribution of anti-TG ab (as measured by EIA) in various thyroid diseases.

Fig. 2: Distribution of anti-TPO ab (as measured by EIA) in various thyroid diseases.

A good correlation was noticed for both antibodies obtained by AT and EIA methods, however the correlation coefficient was better for anti-TPO ab ($r=0.85$, $p<0.01$) than for anti-TG ab ($r=0.66$, $p<0.01$).

DISCUSSION

Attempts to define cut off values for anti-TG ab and anti-TPO ab to differentiate between normal and autoimmune thyroid disease have been described in several reports (2, 3, 7) but only few authors correlated histopathological findings in thyroid tissue with the measured thyroid antibody titers in the serum (8, 9). We studied the cut off points for classification according to clinical studies, by analysing antibody levels in normal subjects and in patients with various thyroid disorders.

Figures 1 and 2 establish the efficiency of the EIA in diagnosing Hashimoto's thyroiditis and the superiority of anti-TPO ab (>550 IU/ml in 90% of patients). Even though there is a significant elevation of anti-TPO ab amongst 60% patients (9/

15) with thyroid carcinoma, the antibody levels do not overlap with those found in Hashimoto's thyroiditis. Since patients with Graves' disease are biochemically toxic and have other clinical features, high antibody levels do not influence the diagnosis except that normal antibodies levels should make one think of a genetic cause for hyperthyroidism (10). Occasionally patients with non autoimmune thyroid disorders have elevated anti-TPO ab, but such patients need an FNAC to confirm or exclude the presence of associated Hashimoto's thyroiditis. Hashimoto's thyroiditis and thyroid cancer can coexist (11,12) and even if a patient has high anti-TPO ab levels, if clinical examination suggests malignancy such patients will need FNAC and surgery if required. Anti-TG ab are elevated in 87% of patients with thyroid carcinoma. The values overlap with those in Hashimoto's thyroiditis and hence are unreliable for discrimination. This is probably because the elevated circulating thyroglobulin levels in thyroid cancer may provoke the production of anti-TG ab. The presence of anti-TG ab in thyroid cancer patients can interfere with quantitative estimation of thyroglobulin which is used as a tumour marker in thyroid cancer (13). The improved sensitivity of the EIA for anti-TG ab is likely to be useful for screening samples from cancer patients before performing estimation of thyroglobulin.

In conclusion the fully automated EIA is a very reproducible, reliable and rapid test for diagnosing Hashimoto's thyroiditis and gives similar results compared with AT titres. Anti-TPO ab as a single test is more specific and cost effective for the diagnosis of autoimmune thyroid disease and measurement of anti-TG ab may be redundant. In patients with thyroid cancer in whom anti-TG ab may interfere with estimation of thyroglobulin. EIA offers a more sensitive method for determining the presence or absence of anti-TG antibody.

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