

Thyroid Stimulating Hormone Receptor Antibody Levels in Singaporean Patients with Autoimmune Thyroid Disease

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Abstract

Stimulating thyrotrophin receptor antibodies (TRAbs) have been identified as the antibodies responsible for the pathogenesis of Graves' disease (GD) while blocking TRAbs have been implicated as the cause of hypothyroidism in some patients with chronic lymphocytic thyroiditis (CLT). TRAb positivity in patients with other thyroid disorders such as silent thyroiditis, toxic multinodular goitre and subacute thyroiditis has been reported but the role of TRAb in these disorders is unclear. A study was carried out to determine the prevalence of TRAb positivity in Singaporean patients with a spectrum of thyroid diseases. TRAb levels were measured in 181 patients with GD, 54 patients with CLT (37 goitrous and 17 agoitrous), 16 patients with thyroid nodules, 11 patients with subacute thyroiditis, 1 patient with hyperthyroidism due to a human chorionic gonadotrophin (HCG)-secreting tumour, 2 patients with thyroid stimulating hormone-secreting tumours and 2 patients with amiodarone-induced dysthyroidism. Using a cut-off of 10.0 U/L, TRAb levels were found to be positive in 79.0% of GD patients, 9.2% of CLT patients (euthyroid and hypothyroid) and no patients with other thyroid disorders. TRAb was a more sensitive marker of GD than anti-thyroglobulin antibodies (53.2%) but not anti-microsomal (78.3%) antibodies. TRAb levels >10.0 U/L appear to be highly specific for autoimmune thyroid disease.

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Introduction

Graves' disease is the commonest cause of thyrotoxicosis amongst Singaporean patients affecting 1.28% of the population.¹ Thyrotrophin receptor antibodies (TRAbs) have been identified as the antibodies responsible for the pathogenesis of GD.² In 1956, Adams and Purves³ made the initial observation which later led to the discovery of long-acting thyroid stimulator (LATS).⁴ LATS was a substance found in GD patients which was distinct from thyroid stimulating hormone (TSH) and was able to stimulate the thyroid glands of guinea-pigs and mice. In 1964, LATS was found to be an immunoglobulin.⁵ Subsequent reports showed that this immunoglobulin could bind to epitopes on the thyrotrophin (TSH) receptor.⁶ In the majority of cases, the binding of TRAbs to the TSH receptor results in activation of the adenylate cyclase system leading to thyroid hormone production and the clinical features of Graves' hyperthyroidism.⁷ These TRAbs are known as stimulat-

ing TRAbs. Less commonly, TRAbs bind to the TSH receptor and inhibit TSH action thereby causing hypothyroidism.⁸⁻¹¹ These antibodies are called blocking TRAbs.

Most commercial laboratories use a TSH binding inhibitory immunoglobulin assay (TBII) to quantitate TRAb levels. This assay measures the ability of the TRAbs to block TSH binding to receptor sites.¹² Using TBII assays, the sensitivity of TRAb in the diagnosis of GD has been reported to range from 43% to 100%.^{13,14} Although TRAb levels are not routinely ordered in cases of thyrotoxicosis, their measurement may be useful in situations where the diagnosis is in doubt. Reported values for the prevalence of blocking TRAb in primary hypothyroidism range from 0% to 75%.¹⁵⁻¹⁸ The significance of TRAb positivity in these patients is that spontaneous euthyroidism or even hyperthyroidism may ensue due to the disappearance of TRAb or when TRAbs switch from a blocking to stimulating¹⁹⁻²¹ type.

The aims of our study were: 1) to determine the sensi-

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tivity and specificity of TRAb in the diagnosis of GD and 2) to document the prevalence of TRAb in patients with goitrous and non-goitrous CLT and to study if this would affect outcome.

Materials and Methods

TRAb levels were measured in 181 patients with GD, 54 patients with CLT, 16 patients with thyroid nodules, 11 patients with subacute thyroiditis, 1 patient with hyperthyroidism due to a human chorionic gonadotrophin (HCG)-secreting tumour, 2 patients with TSH-secreting tumours and 2 patients with amiodarone-induced dysthyroidism. Of the GD patients, 123 patients were newly diagnosed and 58 had relapsed. The diagnosis of GD was based on the clinical features, elevated free thyroxine (fT4) and suppressed TSH levels. In the absence of classical features, technetium scans were performed to exclude silent thyroiditis. TRAb levels were measured while patients were toxic and prior to instituting treatment. Of the patients with CLT, 37 were hypothyroid and 17 were euthyroid. The diagnosis of CLT was made based on histological findings and/or the presence of anti-microsomal antibodies in patients presenting with goitres, thyroid nodules or hypothyroidism. In 37 patients, a goitre was clinically evident and 17 patients were agoitrous. Subacute thyroiditis was diagnosed in 11 patients who presented with classical symptoms and were found to have low uptake on radioiodine scans. Nine were thyrotoxic and 2 were euthyroid at the time of assessment. Of the 16 patients with nodules, 9 had multinodular goitres and 7 had solitary thyroid nodules. All were euthyroid.

TRAb levels were quantified using a radioimmunoassay measuring TSH binding inhibitory activity (RSR, Cardiff). Anti-microsomal antibodies and anti-thyroglobulin antibodies were measured by haemagglutination methods. Tests of significance were done using unpaired t-test on normalised data. P values of less than 0.05 were considered significant. All statistical analyses were done using Minitab Version 10.0 (PA, USA)

Results

TRAb levels in 100 normal controls did not exceed 3.4

U/L (unpublished data). Using this as a reference value, 95.6% of GD patients had elevated values (Table I). Twenty per cent of patients with CLT, 18.2% with subacute thyroiditis and 12.5% with thyroid nodules had levels above this limit. Amongst the 32 patients with thyroid disorders of non-autoimmune aetiology, the highest level was documented in a patient with a HCG-secreting tumour. The TRAb level in this patient was 8.9 U/L. Using a cut-off of 10.0 U/L, TRAb levels were elevated in 143 GD (79.0%) patients. TRAb levels were 36.59 ± 4.10 U/L (mean $\pm$ SE) in new GD patients and 45.32 ± 6.48 in relapsed patients respectively ($P = 0.26$).

78.3% of GD patients were positive for anti-microsomal antibodies and 53.2% were anti-thyroglobulin antibody positive (Table II). 82.4% of anti-microsomal antibody negative patients had positive TRAb levels and 77.8% of TRAb negative patients were anti-microsomal antibody positive. Only 3.6% of GD patients were negative for all 3 autoimmune markers. Of the 54 CLT patients, 17 were euthyroid and 37 were hypothyroid. All euthyroid patients were TRAb negative while 5 (13.5%) hypothyroid patients were TRAb positive. When positive, TRAb levels were markedly elevated (mean $\pm$ SE = 244.8 ± 78.2). TRAb levels remained high even after euthyroidism was achieved with L-thyroxine therapy thus excluding false-positive elevations of TRAb due to TSH. Of these 5 patients, 2 had goitres and 3 were

TABLE II : THYROID AUTOIMMUNE MARKERS IN VARIOUS DISORDERS

Condition	No. of patients	% TRAb >10.0 U/L	% AMA positive	% ATA positive
Graves' disease	181	79.0 %	78.3 %	53.2%
CLT	54	9.2 %	93.7 %	65.6 %
Subacute thyroiditis	11	0 %	9.1%	27.2 %
Thyroid nodules	16	0 %	6.0%	6.0 %
Others	5	0 %	0.0 %	0.0 %

TRAb: thyrotrophin receptor antibodies

AMA: anti-microsomal antibodies

ATA: anti-thyroglobulin antibodies

CLT: chronic lymphocytic thyroiditis

TABLE I: TRAb LEVELS IN VARIOUS THYROID DISORDERS

Condition	No. of patients	% TRAb >3.4 U/L	% TRAb >10.0 U/L	TRAb (mean $\pm$ SE)	TRAb (median)	TRAb (range)
Graves' disease	181	95.6 %	79.0 %	39.43 ± 3.48	26.5	1.4-294.3
CLT	50	20.0 %	9.2 %	24.05 ± 8.22	1.5	0-405.0
Subacute thyroiditis	11	18.2 %	0 %	1.98 ± 0.65	1.5	0-7.3
Thyroid nodules	16	12.5 %	0 %	1.96 ± 0.50	1.3	0-7.2
Others	5	60.0 %	0 %	4.22 ± 1.53	3.5	0-8.9

TRAb: thyrotrophin receptor antibodies

CLT: chronic lymphocytic thyroiditis

agoitrous. They were followed up for 12 months and all patients remained hypothyroid during this period. No patients with nodular thyroid disease, subacute thyroiditis or thyrotoxicosis due to TSH-secreting tumours, HCG-secreting tumours or amiodarone had TRAb levels exceeding 8.9 U/L.

Discussion

TRAbs, while classically associated with GD, have also been detected in patients with chronic lymphocytic thyroiditis,¹⁶ silent thyroiditis,²² toxic multinodular goitre¹⁸ and subacute thyroiditis.²³ In general, two different assay procedures for TRAb measurement are available: 1) An *in vitro* bioassay measuring the ability of the antibodies to stimulate cAMP production by the thyroid cells in culture or 2) A radioimmunoassay measuring the ability of the antibodies to compete with TSH for receptor binding.

The bioassay is often referred to as the thyroid stimulating antibody or immunoglobulin assay (TSAb or TSI) while the radioimmunoassay is known as the TSH binding inhibitory immunoglobulin (TBII) assay. The TSI assay is more sensitive and specific but is technically more difficult and is usually reserved for research purposes. The TBII assay is readily available in commercial laboratories.

TRAb levels are not routinely measured in cases of thyrotoxicosis but may be useful in a number of situations: 1) diagnostic purposes, 2) prognostication of relapse and 3) prognostication of neonatal thyroid disease.

Up to 30% of cases of thyrotoxicosis may be due to causes other than GD.²⁴ Although toxic multinodular goitres,¹⁸ silent thyroiditis²³ and subacute thyroiditis²⁴ have been reported to cause elevated levels TRAb levels, the prevalence of TRAb positivity is much higher in GD.¹⁸ TRAb values in 100 normal controls had previously been established and ranged from 0 to 3.4 U/L. 95.6% of our GD patients and 20.0% of CLT patients were found to have TRAb levels above this limit but 7 patients without autoimmune thyroid disease also had elevated values. Of these, 2 patients had subacute thyroiditis and there was a case each of multinodular goitre, follicular adenoma, TSH-secreting tumour, HCG-secreting tumour and amiodarone-induced thyrotoxicosis.

TRAb levels, therefore, may be mildly elevated in various thyroid disorders such as thyrotoxicosis due to non-thyroidal tumours. However, TRAb levels greater than 10.0 U/L appear to be highly specific for autoimmune thyroid disease.

Other markers of autoimmune thyroid disease include anti-microsomal or thyroid peroxidase antibodies and anti-thyroglobulin antibodies. We found that TRAb was a more sensitive marker of GD than anti-thyroglobu-

lin antibodies and was comparable to anti-microsomal antibodies. When all the 3 markers were used, the sensitivity increased to 96.4%.

In addition to diagnostic purposes, TRAb levels may also be useful for prognostication. Patients with negative TRAb levels may have a milder form of disease²⁵ and consequently better prognosis. TRAb levels at the end of a course of anti-thyroid drug treatment may predict relapse.²⁶⁻²⁸ There is 65% less risk of relapse when TRAb is negative at the end of treatment compared to TRAb-positive patients.²⁶

TRAb levels in the third trimester of pregnancy in GD patients predict neonatal hyperthyroidism.^{29,30} When present in high titre in pregnant females, these antibodies can cross the placenta, bind to the fetal thyroid TSH receptor and cause intra-uterine and neonatal thyrotoxicosis. In patients rendered euthyroid by surgery or radioiodine, TRAbs may persist so that their fetuses may still be at risk despite maternal euthyroidism. If maternal TRAbs are of the blocking type, neonatal hypothyroidism may result.³¹ It is important to be aware of this entity as hypothyroidism in these neonates is transient.

In chronic lymphocytic thyroiditis, hypothyroidism occurs by one of two mechanisms: 1) destructive processes and 2) blocking TRAbs which bind to the TSH receptor and prevent the action of TSH. TRAb positivity in primary hypothyroid patients has been reported to range from 0% to 75%. Our findings concurred with previous reports that CLT patients with blocking TRAbs are invariably hypothyroid. Some authors have observed that these patients may become euthyroid or even hyperthyroid either due to the disappearance of TRAbs or if the TRAbs, for unknown reasons, switch from a blocking to a stimulating type. All our 5 patients remained euthyroid during the period of observation but the duration of follow-up was only one year.

Conclusions

Patients with thyroid nodules, subacute thyroiditis and thyrotoxicosis from a variety of causes other than GD may have mild elevations of TRAb levels above the normal limit of 3.4 U/L. However, levels greater than 10.0 U/L are only seen in autoimmune thyroid disease. Using this level as a cut-off, 79.0% of patients with GD were TRAb positive. TRAb is as sensitive a marker of GD as anti-microsomal antibodies and more sensitive than anti-thyroglobulin antibodies. 9.2% of CLT patients were found to have TRAb values >10.0 U/L. All of these patients were hypothyroid and remained hypothyroid after one year of L-thyroxine therapy. Despite reports of TRAb positivity in a number of thyroid disorders, TRAb levels >10.0 U/L were seen only in patients with GD and CLT in our study. We concluded that TRAb levels above this limit are highly specific for autoimmune thyroid disease and exclude other causes of dysthyroidism.

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