Thyroid peroxidase antibodies in children with autoimmune thyroiditis

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Abstract

Aims: To compare the prevalence of thyroid peroxidase antibodies in 25 children with autoimmune thyroid disorders and in 41 children and young adults with type 1 diabetes, and to test the prevalence of thyrotropin receptor antibodies.

Methods: Two commercially available radioimmunoassays for antibodies to thyroid peroxidase, a commercially available agglutination test of particles coated with thyroid microsomal antigens, and a radioimmunoassay for thyrotropin receptor antibodies were used. Patients and controls were studied.

Results: One of the radioimmunoassays detected thyroid peroxidase antibodies not only in all children with autoimmune thyroid disorders and children and young adults with type 1 diabetes and thyroid microsomal antibodies, but also in 20% of healthy control children without microsomal antibodies. With this thyroid peroxidase assay and with microsomal agglutination, 94% of the children with autoimmune thyroiditis, 71% of those with Graves' disease, and over 90% of those with type 1 diabetes and thyroid dysfunction tested positive. In the other radioimmunoassay for thyroid peroxidase antibodies thyroid peroxidase antibody titres in half or more of the children with microsomal antibodies failed to reach the level of positivity given by the producers. Eighty five per cent of children with Graves' disease and 71% of those with autoimmune thyroiditis had thyrotropin receptor antibodies but so did 35% of children studied for other endocrinological disorders such as delayed growth or puberty.

Conclusions: Testing patients with well characterised disorders of thyroid function and with other endocrine disorders is important in evaluating the efficacy of new diagnostic tests for thyroid auto-antibodies.

Thyroid autoantibodies, markers for autoimmune thyroid disorders, are of many specificities. They are found not only in the serum samples of patients with autoimmune thyroiditis but also in some patients with no clinical or biochemical signs of thyroid dysfunction.¹⁻³ Thyroid microsomal antibodies tend to correlate better with thyroid disorders than do thyroglobulin antibodies.¹⁻³ The test commonly used in assaying thyroid micro-

somal antibodies is agglutination of particles coated with human thyroid microsomes. Most of the thyroid microsomal antibodies are directed against the enzyme thyroid peroxidase, thought to be the major antigen of thyroid microsomes.^{4,5} The prevalence of thyroid peroxidase antibodies has been studied, and thyroid peroxidase has been shown to contain many antigenic determinants.⁶⁻⁹ Commercial radioimmunoassays for assaying anti-thyroid peroxidase are now available and their performance has been tested in adults,¹⁰⁻¹² but not in children. We tested serum samples from children with autoimmune thyroid disease and type 1 diabetes using thyroid microsomal agglutination and two different thyroid peroxidase antibody radioimmunoassays. Sera from other children treated at the Clinics for Endocrinological Disorders and healthy controls were also tested. Thyroglobulin and thyrotropin receptor antibodies, as well as thyroid function, were also determined.

Methods

The patients comprised children with autoimmune thyroid disorders (autoimmune thyroiditis or Graves' disease), children treated at the Clinics for Endocrinological Disorders, and children with insulin dependent diabetes mellitus (IDDM) of recent onset (table 1). Young adolescents with IDDM of about 12 years' duration with or without thyroid dysfunction were also studied. As controls, children admitted to hospital because of minor surgical operations or psychosomatic disorders were also tested. Tests on thyroid function were performed in all but the controls. Informed consent was obtained from the patients or the parents, and the study was approved by the ethical committee at Aurora Hospital.

TESTS ON THYROID FUNCTION

Serum concentrations of thyrotropin and T_4 were determined by radioimmunoassay (TSH-riagnost-hTSH, Behringwerke Inc., Marburg, Germany; T_4 -RIA Becton-Dickinson Inc., Orangeburg, New York) and that of free T_4 (fT_4) as previously described.^{13,14} The normal values were: T_4 , patients younger than 12 years 65-175 nmol/l, older than 12 years, 65-160 nmol/l; fT_4 , 10-25 μ mol/l; thyrotropin, younger than 12 years, 0.5-6 mU/l and older than 12 years, 0.2-4.0 mU/l. The size of thyroid glands was estimated by palpation or by ultrasonography.

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Table 1 Disease profiles of patients studied

Patients	n	Mean age, years (range)	Sex, F/M
Autoimmune thyroiditis	18	13.4 (7.1–16.6)	16/2
Graves' disease*	7	13.8 (10.4–18.3)	5/2
IDDM-A†	15	11.3 (3.9–15.2)	9/6
IDDM-B	11	23.8 (19.2–29.1)	8/3
IDDM-C	15	21.3 (14.7–28.4)	9/9
Endocrine disorders‡	21	9.4 (0.5–17.4)	11/10
Controls	20	8.5 (2.1–14.8)	12/8

†Type 1 diabetics; tested within two weeks of diagnosis (A) or about 12 years after diagnosis (B and C); B with thyroid dysfunction; C with normal thyroid function.

‡Diagnoses comprised: constitutional delay of growth and puberty (n = 7); congenital hypothyroidism (n = 2); toxic multinodular goitre (n = 1); hypoparathyroidism (n = 1); growth hormone neurosecretory dysfunction (n = 2); pubertés praecox (one because of supracellular tumours, one idiopathic); constitutional tall stature (n = 1); hypogonadotropic hypogonadism (n = 1); anorchism (n = 1); Turner's syndrome (n = 1); adrenarche praecox (n = 1); nanism due to fetal alcohol syndrome (n = 1).

ASSAYS OF THYROID MICROSOMAL ANTIBODIES

Antibodies against thyroid microsomal antigens were assayed using agglutination of gelatin particles sensitised with human microsomal antigens (Serodia-AMC, Fujirebio Inc., Tokyo, Jpn) and by two tests based on inhibition of binding of radiolabelled thyroid peroxidase to monoclonal antibodies in solid phase by antibodies in the serum tested (TPO-A, Dynotest, Henning Inc., Berlin, Germany, and TPO-B, AB-TPO, Sorin Inc., Saluggia, Italy). In TPO-A and -B tests serum was diluted as suggested by the manufacturers—that is to 1 in 20 and 1 in 50, respectively, and the units of thyroid peroxidase antibodies were calculated with the aid of the standards provided by the manufacturers. Titres of $\geq 1/400$ were regarded as clinically significant in the agglutination test. In the TPO-A test values of ≥ 100 U and in the TPO-B test values of ≥ 10 U were suggested as being clinically significant by the manufacturers.

ASSAY OF THYROGLOBULIN ANTIBODIES

Antibodies against thyroglobulin were measured by agglutination of gelatin particles coated with human thyroglobulin (Serodia-ATG, Fujirebio Inc., Tokyo, Japan). Agglutination titres 1/100 were considered clinically significant.

ASSAY OF TSH RECEPTOR ANTIBODIES

Radioreceptor assay (TRAK-Assay, Henning Inc., Berlin, Germany) which measures the ability of the patient's serum to inhibit the binding of radiolabelled thyrotropin to solubilised thyrotropin-receptor, was used for the determination of autoantibodies to the thyrotropin receptor. Values of ≥ 15 U/l were considered positive, and those of ≥ 10 but less than

15 U/l considered to be in the "grey-area" of the test.

The Statview program was used to calculate the Spearman correlation coefficients.

Results

Using agglutination, thyroid microsomal antibodies in titres of ≥ 400 were present in 94% of children with autoimmune thyroiditis (table 2), in 71% of children with Graves' disease, and in 91% of type 1 diabetic young adults treated for thyroid dysfunction (IDDM-B). The prevalence of microsomal antibodies in children with newly diagnosed IDDM (IDDM-A) and young adults with IDDM but without thyroid dysfunction (IDDM-C) was 20% and 13%, respectively. None of the control children had thyroid antibodies with this test, while one of the children with an endocrinological disorder, Turner's syndrome, did with a titre of 1/400. High titres of microsomal antibodies were only seen in patients with autoimmune thyroiditis or with IDDM accompanied by thyroid dysfunction: 28% and 27%, respectively, of these patients had microsomal antibodies in titres of ≥ 6400 , and 11% compared with 18% in titres of $\geq 25\ 000$.

In the thyroid peroxidase test A (TPO-A) 94% of the children with autoimmune thyroiditis, 71% of those with Graves' disease, 20% of children with newly diagnosed type 1 diabetes, all young adults with IDDM and concomitant thyroid disorders, and one of those with an endocrinological disorder (Turner's syndrome) had thyroid peroxidase antibodies in concentrations of ≥ 100 U/ml (suggested limit for positivity) but so did 20% (4/20) of normal controls and 50% of patients with longstanding IDDM but without thyroid problems (table 3). Concentrations of ≥ 1000 U/ml were found in 61% of children with autoimmune thyroiditis, in 14% of the children with Graves' disease, in 71% of adolescents with IDDM and thyroid dysfunction, and up to 25% of other diabetics. The proportions of patients with autoimmune thyroiditis or IDDM together with thyroid dysfunction having $\geq 10\ 000$ U/ml of thyroid peroxidase antibodies were 17% and 29%, respectively. The correlation between microsomal antibody titre and the thyroid peroxidase antibody units given by TPO-A test was 0.874.

In the thyroid peroxidase test B (TPO-B) 50% of the children with autoimmune thyroiditis had antibodies of ≥ 10 U/ml (suggested limit for positivity) as did 29% of the

Table 2 Presence of thyroid microsomal antibodies detected by agglutination

Patients	Number (%) with antibody titres of					
	<100	≥ 100	≥ 400	≥ 1600	≥ 6400	$\leq 25\ 000$
Autoimmune thyroiditis	1/18 (6)*	17/18 (94)	17/18 (94)	12/18 (67)	5/18 (28)	2/18 (11)
Graves' disease	2/7 (29)	5/7 (71)	5/7 (71)	3/7 (43)	0/7 (0)	0/7 (0)
IDDM-A	12/15 (80)	3/15 (20)	3/15 (20)	2/15 (13)	0/15 (0)	0/15 (0)
IDDM-B	0/11 (0)	11/11 (100)	10/11 (91)	8/11 (73)	3/11 (27)	2/11 (18)
IDDM-C	12/15 (80)	3/15 (20)	2/15 (13)	1/15 (7)	0/15 (0)	0/15 (0)
Endocrinological disorders	20/21 (95)	1/21 (5)	1/21 (5)	0/21 (0)	0/21 (0)	0/21 (0)
Controls	20/20 (100)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)

*Number (%) with antibodies at the dilution given.

Table 3 Thyroid peroxidase antibodies detected by TPO-A test (U/ml)

Patients	<100	≥100	≥200	≥1000	≥10 000
Autoimmune thyroiditis	1/18 (6)	17/18 (94)	16/18 (89)	11/18 (61)	3/18 (17)
Graves' disease	2/7 (29)	5/7 (71)	4/7 (57)	1/7 (14)	0/7 (0)
IDDM-A	12/15 (20)	3/15 (20)	3/15 (20)	2/15 (13)	0/7 (0)
IDDM-B	0/7 (0)	7/7 (100)	6/7 (86)	5/7 (71)	2/7 (29)
IDDM-C	8/16 (50)	8/16 (50)	5/16 (31)	4/16 (25)	1/16 (6)
Endocrinological disorders	20/21 (95)	1/21 (5)	0/21 (0)	0/21 (0)	0/21 (0)
Controls	16/20 (80)	4/20 (20)	0/20 (0)	0/20 (0)	0/20 (0)

Table 4 Thyroid peroxidase antibodies detected by TPO-B test (U/ml)

Patients	<2.5	≥2.5	≥100	≥≠20
Autoimmune thyroiditis	2/18 (11)	16/18 (89)	9/18 (50)	4/18 (22)
Graves' disease	3/7 (43)	4/7 (57)	2/7 (29)	0/7 (0)
IDDM-A	12/15 (80)	3/15 (20)	2/15 (13)	0/15 (0)
IDDM-B	1/7 (14)	6/7 (86)	4/7 (57)	2/7 (29)
IDDM-C	11/16 (69)	5/16 (31)	2/16 (13)	1/16 (6)
Endocrinological disorders	21/21 (100)	0/21 (0)	0/21 (0)	0/21 (0)
Controls	20/20 (100)	0/20 (0)	0/20 (0)	0/20 (0)

Table 5 Thyrotropin receptor antibodies (U/ml)

Patients	<10	≥10	≥15
Autoimmune thyroiditis	1/14 (7)	13/14 (93)	10/14 (71)
Graves' disease	0/7 (0)	7/7 (100)	6/7 (85)
IDDM-A	15/15 (100)	0	0
IDDM-B	7/7 (100)	0	0
IDDM-C	9/9 (100)	0	0
Endocrinological disorders	5/20 (25)	15/20 (75)	7/20 (35)
Controls	20/20 (100)	0	0

children with Graves' disease, and 57% of young adults with IDDM and thyroid disease (IDDM-B), and 13% of the other type 1 diabetics, while none of the others had ≥10 U/ml of thyroid peroxidase antibodies (table 4). The correlation between titres of microsomal antibodies and Units/ml in the TPO-B test was 0.867 and that between the units given by TPO-A and -B, 0.892.

Thyroglobulin antibodies were detected in seven patients: three children with Graves' disease (43%), two with autoimmune thyroiditis (11%), one with Turner's syndrome and in one young adult with longstanding IDDM. All of them also had microsomal antibodies and thyroid peroxidase antibodies in the TPO-A test, but the TPO-B test failed to detect antibodies in two.

Thyrotropin receptor antibodies were detected in 71% of the children with autoimmune thyroiditis, in 85% of children with Graves' disease, and in 35% of children with other endocrinological disorders in concentrations of ≥15 U/l, and in 93%, 100%, and 75%, respectively, in concentrations of ≥10 U/l (table 5).

Three of the 18 children with autoimmune thyroiditis were hypothyroid and receiving thyroxine; all the children with Graves' disease were receiving carbimazole and euthyroid, and the young adults with IDDM and thyroid dysfunction as well as the two children with congenital hypothyroidism were also receiving thyroxine and euthyroid. The rest had normal

concentrations of thyrotropin and T₄ and were clinically euthyroid. The titres of the antibodies tested showed no correlation with the concentrations of TSH or T₄.

Discussion

The autoimmune aetiology of biochemically and clinically diagnosed thyroid dysfunction is usually confirmed by the finding of thyroid autoantibodies. If thyroid antibodies cannot be detected the diagnosis of autoimmune thyroiditis can be confirmed by fine needle aspiration biopsy, a procedure usually required in less than one tenth of the patients.^{3 13 14}

The tests for thyroid autoantibodies currently available are assays of thyroid microsomal, thyroid peroxidase, thyroglobulin, and thyrotropin receptor antibodies. In the patients described here thyroglobulin antibodies were rarely detected and only with microsomal antibodies. The prevalence of thyroglobulin antibodies in different patient groups was also similar to that previously reported.^{3 13 14}

A comparison of the two thyroid peroxidase tests and the agglutination test routinely used to detect thyroid microsomal antibodies (table 6) showed that if the levels of positivity of the thyroid peroxidase tests given by the manufacturers—that is, 100 and 10 U/ml were used, one (TPO-A) would detect antibodies in patients with thyroid dysfunction and those with IDDM as often as the agglutination test, but 20% of the controls would also be positive; the other (TPO-B) would detect about half of the patients with microsomal antibodies by agglutination. If the levels of positivity were adjusted to ≥200 U/ml in the TPO-A and to ≥2.5 U/ml in the TPO-B test the number testing positive in the three tests would be similar. Up to 98% of adults with autoimmune thyroid disorders, and up to 20% of normal adults have been shown to have anti-TPO antibodies by the TPO-A test.¹⁰⁻¹² The TPO-A test has not been used to detect microsomal (TPO) antibodies in children but in a commercially available enzyme linked immunosorbent assay for thyroid peroxidase 8% of newly diagnosed children with IDDM and 2% of the control children had thyroid peroxidase antibodies.¹⁵

High titres of thyroid peroxidase antibodies (≥1000 U/ml) by the TPO-A test were found more frequently in IDDM and in thyroid dysfunction than high titres of microsomal antibodies (≥6400). Whether thyroid peroxidase antibodies would be more sensitive in predicting the development of thyroid dysfunction remains to be established. The reason for the different sensitivities of the TPO-A and -B tests could be that different epitopes were

Table 6 Comparison of agglutination, TPO-A, and TPO-B tests

Test	Percentage positive				
	Autoimmune thyroid disorders	Graves' disease	IDDM	Endocrinological disorders	Controls
Agglutination titre of ≥400	94	71	37	5	0
TPO-A of ≥200/≥100* IU	89/94	57/71	36/46†	0/5	0/20
TPO-B of ≥2.5/≥10* IU	89/50	57/29	37/21‡	0/0	0/0

*Positivity levels suggested by the manufacturers.

†14/39 (18/39); ‡14/38 (8/38).

exposed on thyroid peroxidase preparations used in the tests, a fact which may affect the reactivity and perhaps define different subgroups of patients.¹⁶⁻¹⁸

The proportion of children with autoimmune thyroiditis and Graves' disease with thyrotropin receptor antibodies in titres of ≥ 15 U/l was 71% and 85%, respectively. Up to 98% of adults with Graves' disease and less than 10% of adults with autoimmune thyroiditis have been shown to have thyrotropin receptor antibodies by the test used here.^{3 19 20} Results using this test have not been reported in children before but other tests have detected these antibodies in up to 98% of children with Graves' disease and, depending on the assay, in none or up to 70% of children with autoimmune thyroiditis.²¹⁻²⁴

None of the patients with type 1 diabetes, whether with thyroid dysfunction or with microsomal/thyroid peroxidase antibodies, had thyrotropin receptor antibodies. The outcome of the autoimmune thyroiditis in children with diabetes differs from that of autoimmune thyroiditis in children without diabetes. Two thirds of the children with diabetes and thyroiditis become hypothyroid as opposed to one third of the children with thyroiditis alone.^{13 14} This suggests that the mechanism(s) involved in the development of thyroid dysfunction in children with type 1 diabetes may differ from those in children with autoimmune thyroiditis.

One third ($n = 7$) of the control children tested positive in the anti-thyrotropin receptor assay at the level of ≥ 15 U/l, and 75% at the level of ≥ 10 U/l. We do not know the reason why. The reactivity may have been due to non-specific binding,^{25 26} or perhaps due to antibodies against other hormones sharing the alpha chain of thyrotropin. Three of these children were or had been receiving testosterone, two growth hormone and two thyroxine. Further studies are required to resolve the specificity of this reactivity.

Analysis of thyroid autoantibodies in patients with well characterised disorders of thyroid function and with other endocrine disorders is useful in establishing the value of tests for organ specific circulating antibodies. Whether more sensitive and specific methods—for instance, using cloned peptide fragments of autoantigens^{27 28}—might be useful in predicting the development of disorders of thyroid function or in differentiating between different clinical forms of thyroid dysfunction remains to be established. The reason(s) for reactivity in the thyrotropin receptor assay used here need to be studied in detail.

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