CLINICAL RESEARCH

Detection of familial dysalbuminaemic hyperthyroxinaemia

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Abstract

A simple test of *in vitro* thyroxine binding to serum proteins was used to screen serum samples from euthyroid patients with unexplained increases in the free thyroxine index. A diagnosis of familial dysalbuminaemic hyperthyroxinaemia was presumed in 14 unrelated subjects and six first degree relatives. Increased binding of thyroxine to thyroxine binding prealbumin was diagnosed in one woman with four unaffected relatives. Seven patients with familial dysalbuminaemic hyperthyroxinaemia had been treated for presumed thyrotoxicosis: two had typical Graves' disease and one subacute thyroiditis. Four other patients had been mistakenly treated with radioactive iodine or antithyroid drugs. In previously treated patients familial dysalbuminaemic hyperthyroxinaemia was suspected from the combination of a high serum thyroid stimulating hormone concentration and a normal but invalid free thyroxine index.

Physicians should be cautious in accepting a diagnosis of thyrotoxicosis based mainly on a raised serum thyroxine concentration.

Introduction

A raised serum total thyroxine concentration or free thyroxine index may be mistaken for thyrotoxicosis in the syndrome of familial dysalbuminaemic hyperthyroxinaemia,¹⁻³ an autosomal dominant syndrome resulting from unusual, high capacity binding of thyroxine to serum albumin. In this syndrome serum free thyroxine concentrations tested by equilibrium dialysis, serum

triiodothyronine concentration, and thyrotrophin releasing hormone responses are normal and affected subjects are usually euthyroid.

Subjects with this protein binding variant may also present with an apparently normal free thyroxine index in the presence of hypothyroidism induced by radioactive iodine or treatment with antithyroid drugs. In this report we describe the diagnostic usefulness of a simple *in vitro* test of serum thyroxine binding.⁴

Patients and methods

We studied 42 patients (eight men and 34 women), aged 17-72 years, referred to the thyroid clinic for assessment of possible thyrotoxicosis diagnosed on the basis of a raised free thyroxine index. Serum samples from 32 of these patients were further analysed because of the combination of a raised serum free thyroxine index and a normal thyrotrophin releasing hormone response. In the 10 other patients a change in the normal relation between basal serum thyroid stimulating hormone concentration and free thyroxine index had been observed. Seven of the 42 patients had been treated for thyrotoxicosis.

We measured the basal serum free thyroxine index, free triiodothyronine index, thyroid stimulating hormone concentrations, and the response of thyroid stimulating hormone to stimulation with thyrotrophin releasing hormone, as previously defined and reported.⁵ Basal thyroid stimulating hormone concentrations were also measured by radioimmunoassay modified to increase sensitivity to 0.1 μ U/ml; results from this assay correlated well with the increment in thyroid stimulating hormone after thyrotrophin releasing hormone stimulation ($r=0.92$) and similarly distinguished normal subjects from patients with subnormal thyroid stimulating hormone concentrations and definite thyrotoxicosis.⁶ In 11 subjects with familial dysalbuminaemic hyperthyroxinaemia the serum free thyroxine concentration was also estimated directly by both Amerlex (Amersham) and Gammacoat two step (Clinical Assays) kit methods.

SERUM THYROXINE BINDING TESTS

The binding of thyroxine labelled with iodine-125 (¹²⁵I) to serum proteins was estimated by a modified version of our *in house* triiodothyronine resin uptake method using heat denatured macroaggregated albumin as the adsorbent. Bovine serum albumin (0.25% in acetate buffer (0.3M, pH 5.5) was denatured by heating at 80°C for one hour. The particles of macroaggregated albumin were separated by centrifugation, resuspended in 50 ml trometamol (TRIS) saline buffer (0.05M, pH 7.5), and then aspirated through a 20 g

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needle to produce particles of a uniform size (approximately 10 μ m in diameter). Working reagent was prepared by diluting the solution of macroaggregated albumin with 500 ml trometamol saline buffer, adding cold thyroxine to a concentration of 1.3 nM and 125 I labelled thyroxine (high specific activity, Amersham) to 40 000 cpm.

To perform the assay 10 μ l serum was incubated with 1 ml of this working reagent for 10 minutes at room temperature, centrifuged, and decanted and the 125 I labelled thyroxine uptake of the macroaggregated albumin was counted. The reagent volume was adjusted with buffer such that normal serum gave an uptake of roughly 20% of the added counts. The uptake of thyroxine loaded macroaggregated albumin was obtained by repeating the test as described above in the presence of a 50-fold excess of cold thyroxine by adding 10 μ l of cold thyroxine standard (5 μ M) to the 10 μ l serum sample. For a one to 100 dilution of serum (thyroxine concentration about 10^{-8} M) this gives a final thyroxine concentration of 5×10^{-8} M in the incubation mixture.⁴ In screening for the presence of possible excess thyroxine binding to prealbumin a 1000-fold excess of added cold thyroxine was used to give a final thyroxine concentration of 10^{-6} M.⁷ The results were calculated and expressed as the increment between the percentage basal and thyroxine loaded macroaggregated albumin uptakes of 125 I labelled thyroxine. The unpaired Student's *t* test was used in statistical analysis (table I).

Results

We found anomalous thyroxine binding in the serum of 14 unrelated subjects and six members of four families tested. These patients had been selected for study because of conflicting clinical and laboratory results. The results of the in vitro thyroxine binding tests in these subjects indicated that dysalbuminaemia was the likely cause of the raised free thyroxine index. The free thyroxine index was raised in all 14 untreated subjects (mean 192 (normal 70-145), range 155-337). All 14 appeared to be clinically euthyroid and had a normal free triiodothyronine index. Thyrotrophin releasing hormone stimula-

FIG 1—Relation between serum thyroid stimulating hormone concentrations (TSH) and free thyroxine (T4) index in 33 samples from 19 patients with familial dysalbuminaemic hyperthyroxinaemia. Solid lines enclose range of values in 72 normal subjects and 128 patients with hypothyroidism or thyrotoxicosis. Serum thyroid stimulating hormone values above 6 μ U/ml are from patients with familial dysalbuminaemic hyperthyroxinaemia and hypothyroidism induced by treatment.

TABLE I—Mean (range) results of thyroid function tests in 11 patients with familial dysalbuminaemic hyperthyroxinaemia and 55 normal subjects

	n	Free thyroxine index	Free thyroxine		Free triiodothyronine index	Increment in uptake of 125 I labelled thyroxine (%)
			(Amerlex) (pmol/l)	(Clinical assays) (pmol/l)		
Thyroxine binding anomaly	11	176* (139-242)	30.0* (23-39)	22.2* (18-26)	1.67 (1.3-2.15)	2.5* (0.4-9)
Reference range	55	96.5 (70-145)	17.1 (12.5-23)	17.7 (13-24)	1.72 (1.4-2.5)	25.0 (18-33)

**p* < 0.001 compared with the reference range.

Conversion: SI to traditional units—Thyroxine: 1 pmol/l $\approx$ 78 pg/100 ml.

TABLE II—Mean (SD) results of increment in 125 I labelled thyroxine uptake testing (%) in serum at two thyroxine concentrations

Patients	n	5×10^{-8} M thyroxine	10^{-6} M thyroxine
Normal	55	25 (4.0)	43 (3.0)
FDH	20	2.9 (1.6)	13.2 (1.8)
TBPA	1	13.4	33.8

FDH = familial dysalbuminaemic hyperthyroxinaemia;
TBPA = thyroxine binding prealbumin.

tion tests performed in nine subjects were also normal. Table I compares the free thyroxine index determined with the two free thyroxine kit methods in 11 subjects. Concentrations of free thyroxine were also above the upper limit of normal by the Amerlex and Clinical Assays methods in 10 and four samples respectively.

Table II shows the results of the increment in 125 I labelled thyroxine uptake tests at two thyroxine concentrations. Patients with hypothyroidism, thyrotoxicosis, or high serum thyroxine binding globulin concentrations had uptake values within 2SD of the mean normal value. With the exception of one patient, to be described below, there was no overlap in individual values for incremental uptake of 125 I

labelled thyroxine between normal subjects and patients with the presumed anomaly of thyroxine albumin binding.

In one sample, from a woman with symptomatic anxiety and a free thyroxine index of 170, we obtained an intermediate value for incremental uptake of 125 I labelled thyroxine of 13.3%, which increased to 33.8% at 10^{-6} M thyroxine. This combination suggested the possibility of increased thyroxine binding to thyroxine binding prealbumin. This supposition was confirmed by an immunoprecipitation method in the laboratory of Dr J Stockigt. Immunoprecipitation of 125 I labelled thyroxine from serum by antiserum to human prealbumin was appreciably increased at 42-48% compared with less than 20% in normal subjects. As in the report from Moses *et al*,⁸ the concentration of immunoreactive prealbumin was slightly increased, at 455 mg/l (normal 200-400 mg/l). Four first degree relatives were tested and found to have normal free thyroxine index values and normal in vitro thyroxine binding.

Serum samples from patients with an unexplained increase in free thyroxine index were selected for thyroxine binding studies for several reasons. Firstly, serum samples from 32 subjects who had shown a normal response to thyrotrophin releasing hormone stimulation tests and a raised free thyroxine index were tested. Abnormal thyroxine binding was detected in nine of these 32 subjects. Secondly, anomalous thyroxine binding was detected in five of 10 serum samples taken from patients who showed a shift in the usual inverse relation between serum thyroid stimulating hormone and free thy-

roxine index measurements. Figure 1 illustrates the normal range of this relation with a composite graph of values from 24 patients with untreated primary hypothyroidism, 72 normal subjects, 43 patients with thyrotoxicosis, and 61 patients with hypothyroidism treated with thyroxine. This figure shows 33 values from 19 patients with presumed familial dysalbuminaemic hyperthyroxinaemia. Thyroid stimulating

Figure 2 shows the relation between the free thyroxine index and serum thyroid stimulating hormone values over time for three patients and table III the history and results of thyroid function tests in six patients reported on below.

Case 1, a 50 year old woman, presented with a typical attack of painful subacute thyroiditis, which resolved after a short course of prednisone. An unusual feature was the presence of a high free thyroxine index (440), which remained raised after clinical improvement, prompting a brief trial of carbimazole treatment one year later. Her serum thyroid stimulating hormone concentration rose to over 40 $\mu\text{U/ml}$, but the serum thyroxine concentration remained in the normal range. Normal thyrotrophin releasing hormone responses were obtained on two occasions, and eventually, five years after her initial presentation, the thyroxine binding anomaly was established. One of her three first degree relatives tested was also found to have hyperthyroxinaemia.

Case 2, a 40 year old man, presented with definite thyrotoxicosis including a 10 kg loss of weight associated with diffuse goitre, positive serum antimicrosomal antibodies, and a free thyroxine index of 850. Hyperthyroid Graves' disease was diagnosed, and he was treated with antithyroid drugs and then radioactive iodine with complete resolution of his symptoms and signs. Mild clinical hypothyroidism was treated with thyroxine 0.05 mg daily. In response to this treatment his serum thyroid stimulating hormone concentration fell from 21 to 6.5 $\mu\text{U/ml}$ and his free thyroxine index rose from 160 to 212.

Case 3, a 60 year old woman, presented with symptoms of anxiety and a raised free thyroxine index. She was treated with radioactive iodine, which induced clinical hypothyroidism. While receiving thyroxine 0.15 mg daily her free thyroxine index was 420, but she remained clinically euthyroid. Withdrawal of thyroxine treatment led to a fall in the free thyroxine index to 72 and a rise in the thyroid stimulating hormone concentration to 49 $\mu\text{U/ml}$. When the abnormal serum thyroxine binding was discovered review of her records showed various discrepancies that had been overlooked because of the suggestive clinical features at initial presentation and her subjective clinical response to antithyroid drug treatment. She had had negative serum thyroid autoantibodies, a normal uptake of pertechnetate, and a normal serum free triiodothyronine index. Two of her three children were also found to have familial dysalbuminaemic hyperthyroxinaemia. The most appropriate dose of thyroxine to bring her serum thyroid stimulating hormone concentration back to normal was 0.1 mg daily. With this treatment she was clinically euthyroid, her free thyroxine index was 220-260, and her free triiodothyronine index was in the middle of the normal range.

Case 5—The correct diagnosis in this patient would have remained unsuspected but for the fortuitous discovery of abnormal thyroxine binding in serum taken at a regular treatment clinic. In retrospect, he had not shown supportive symptoms or signs of thyrotoxicosis or Graves' disease. His mother (case 6) had been treated for presumed thyrotoxic Graves' disease in 1968. Review of her medical records still supports that diagnosis. She was studied and found to have familial dysalbuminaemic hyperthyroxinaemia and subclinical hypothyroidism after surgical treatment of Graves' disease.

Discussion

Familial dysalbuminaemic hyperthyroxinaemia is an autosomal dominant syndrome that has been described in 58 subjects from 18 families⁹ since the original report by Henneman *et al* in 1979.¹ Affected members have unusual serum albumin, which shows increased binding affinity for thyroxine but not for triiodothyronine.¹⁰ The increased binding of thyroxine to albumin is therefore not detected by conventional resin triiodothyronine uptake methods, which use isotopically labelled triiodothyronine. White *et al* have developed a simple adsorption test that uses labelled thyroxine and trometamol buffer to assess in vitro thyroxine binding in the presence of excess unlabelled thyroxine.⁴ Using their method we detected dysalbuminaemic hyperthyroxinaemia in the serum of 14 unrelated subjects and six members of four families tested.

We had incorrectly diagnosed thyrotoxicosis in four patients because of our overreliance on a raised free thyroxine index combined with possible symptoms of thyrotoxicosis, particularly anxiety. In two of these patients the development of a goitre and a raised isotopic neck uptake during antithyroid drug treatment were additional diagnostic distractors. Newer methods for

FIG 2—Serial values of serum thyroid stimulating hormone concentration (TSH) (○) and free thyroxine index (FT4I) (●) in three patients (cases 1, 2, and 3) with familial dysalbuminaemic hyperthyroxinaemia.

hormone concentrations over 6 $\mu\text{U/ml}$ were in three patients with hypothyroidism induced by treatment. In one patient the diagnosis was made fortuitously by the screening of all samples from a single thyroid treatment clinic.

Seven patients had received treatment for thyrotoxicosis. Two had been appropriately treated for possible thyrotoxic Graves' disease and one for typical subacute thyroiditis. Four patients had been inappropriately treated, three with radioactive iodine and one with long term antithyroid drugs.

measuring free thyroxine also gave misleading results, as previously described.¹¹ Free thyroxine concentrations measured by equilibrium dialysis, serum triiodothyronine concentrations measured by radioimmunoassay, and results of thyrotrophin releasing hormone stimulation tests are all normal in this syndrome.¹

Other possible causes of euthyroid hyperthyroxinaemia in this group of patients include non-thyroidal illness and the use of

are usually absent in familial dysalbuminaemic hyperthyroxinaemia and will lend support to the presence of true thyrotoxic Graves' disease. A raised serum triiodothyronine concentration seen by radioimmunoassay, an absent thyrotrophin releasing hormone response, or a subnormal serum thyroid stimulating hormone concentration will further exclude familial dysalbuminaemic hyperthyroxinaemia as the sole diagnosis.

The incidence of familial dysalbuminaemic hyperthyroxinaemia

TABLE III—History and results of thyroid function tests in six patients with familial dysalbuminaemic hyperthyroxinaemia

Case No	Sex/Age (years)	Diagnosis and clinical notes	Serum free thyroxine index*	Serum free triiodothyronine index†	Basal TSH (μU/ml)‡	Thyroid antimicrosomal antibodies
1	F 50	Subacute thyroiditis (1977)	440	3.5	UD	Negative
		Carbimazole treatment (1978)	55	1.0	4.0	
		No treatment (1980)	235	1.4	1.3	
2	M 40	Hyperthyroid Graves' disease	850	2.0	21 6.5	1:3200
		Hypothyroid after ¹³¹ I treatment	169			
		Receiving T4 0.05 mg daily	212			
3	F 60	Thyrotoxicosis	213	1.8	42 UD	Negative
		Hypothyroid after ¹³¹ I treatment	83			
		Receiving T4 0.15 mg daily	420			
4	F 45	Thyrotoxicosis	153	2.15	12.4 4.3	Negative
		Hypothyroid after ¹³¹ I treatment	176			
		Receiving T4 0.1 mg daily				
5	M 17	Juvenile Graves' disease	218	1.9	1.0	Negative
		Carbimazole treatment for 8 years				
		Untreated				
6	F 48	Past Graves' disease (mother case 5)	87 183	1.9	4.8 2.2	1:3200
		Partial thyroidectomy, aged 32				
		Untreated				
		Receiving T4 0.1 mg daily				

Normal values: *70-145; †1.4-2.5; ‡0.4-4.0 μU/ml.

TSH = thyroid stimulating hormone; UD = undetected; T4 = thyroxine; ¹³¹I = iodine-131.

drugs that interfere with thyroxine metabolism, such as amiodarone or propranolol.⁹ These causes were easily excluded both by the history and our failure to detect abnormal thyroxine binding in serum from such patients.

The combination of familial dysalbuminaemic hyperthyroxinaemia with thyrotoxicosis delayed complete diagnosis in two patients until we appreciated the importance of an unusually high free thyroxine index after induced hypothyroidism. The anomalous binding of albumin and thyroxine increases the normal total serum thyroxine concentration by about 100 nmol/l (7.8 μg/100 ml).¹⁰ In the present study we observed a comparable increase of 80 nmol/l (6.2 μg/100 ml) in 11 untreated subjects. The shift to the right in the usual inverse relation between thyroid stimulating hormone and free thyroxine index also reflects the increase in serum thyroxine binding (fig 1). Since introducing a modified sensitive serum thyroid stimulating hormone assay to screen for the presence of thyrotoxicosis we have diagnosed two additional patients with familial dysalbuminaemic hyperthyroxinaemia in this way.

Only two reports have previously described hyperthyroxinaemia due to anomalous thyroxine binding prealbumin; a father and son were described by Moses *et al*⁸ and a patient with a glucagonoma producing thyroxine binding prealbumin by Jacobsson *et al*.¹² Our patient, a young woman, presented with anxiety and a raised free thyroxine index. Four first degree relatives were unaffected. The diagnosis was suspected on the basis of the intermediate result for uptake of ¹²⁵I labelled thyroxine combined with the presumed increase in thyroxine binding prealbumin saturation at 10⁻⁶M thyroxine, as predicted by Stockigt *et al*⁷ and confirmed by Moses *et al*.¹³ We have also tested serum from one patient with a glucagonoma with a negative result.

Our report emphasises the need for physicians to be cautious in accepting a diagnosis of thyrotoxicosis based primarily on a raised serum thyroxine concentration obtained in a screening test. Classic features of goitre, exophthalmos, positive serum thyroid antimicrosomal antibodies, and a raised isotopic neck uptake

aemia due to abnormal albumin binding remains unknown. We have diagnosed these anomalies in thyroxine binding over 18 months in subjects drawn from a population of about one million. Our results suggest that the wider use of in vitro thyroxine binding tests will show many further examples of these recently described protein variants.

We thank Dr Jan Stockigt of Melbourne, Australia, for the prealbumin immunoprecipitation studies and helpful discussion. Amerlex kindly donated the Amerlex free thyroxine radioimmunoassay kit. We also acknowledge the support of the New Zealand Medical Research Council.

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(Accepted 9 January 1985)