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Case report

False elevation of serum thyroxine in myxoedema due to thyroxine-binding autoantibodies. A diagnostic pitfall

G P R Archbold, H J Southgate, J D Teale, V Marks

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The presence of anti-thyroid antibodies can cause artefactual estimates of thyroid hormones by radioimmunoassay. We describe a case of myxoedema with spuriously high serum total and 'free' thyroxine (T_4) levels which were initially misleading. Further investigations revealed the presence of IgG autoantibodies to T_4.

CASE HISTORY

A 29-year-old male Caucasian with no relevant family history presented with a history of constipation, increasing tiredness and a feeling of intense coldness. His performance at sport, notably rugby and cricket, had fallen off badly largely because of weakness and muscle stiffness. His hair was falling out and his skin had become very dry. The clinical diagnosis was of myxoedema. He had a past history of nocturnal convulsions related to heavy alcohol consumption for which he was taking phenytoin sodium 100mg at night, but alcohol intake was now modest. Initial tests of thyroid function were surprisingly high — serum total T_4 124nmol/l (reference range 60 – 160), serum 'free' T_4 168pmol/l (reference range 9 – 28). He was referred to St Luke's Hospital, Guildford, for further investigation.

On examination he appeared myxoedematous. Heart rate was regular, 60 beats/min, and pulse was of small volume. Blood pressure was 120/80mmHg. The thyroid gland was not enlarged. Examination of the nervous system and in particular of the tendon reflexes produced normal results. Electromyography suggested a mild proximal myopathy. Motor nerve conduction velocities of the right median and lateral popliteal nerves were in the low normal range at 50 and 34 m/sec respectively; sensory action potential of the median nerve at the wrist on stimulation of the index finger had normal amplitude of 9μV but a prolonged peak latency of 4msec. ECG showed sinus rhythm with low voltage complexes and T-wave inversion.

Department of Clinical Biochemistry, St Luke's Hospital, Guildford, Surrey.
G P R Archbold, BSc, PhD, MRCP, Senior Registrar.
H J Southgate, BSc, MRCP, MRCPath, Senior Registrar.
J D Teale, BSc, PhD, Principal Biochemist.
V Marks, MA, DM, FRCP, PRCPath, Professor.
Correspondence to: Dr G P R Archbold, Department of Clinical Biochemistry, Royal Victoria Hospital, Belfast BT12 6BA.

Detailed thyroid function tests are shown in the Table. Other screening investigations were normal apart from serum creatine kinase 3250U/l (normal <160) and aspartate transaminase 129U/l (normal <40). Serum phenytoin was <10μmol/l. Serum albumin was 47g/l and total plasma protein 83g/l with a normal electrophoretic pattern. Serum IgG, IgM and IgA were all within normal limits. Haemoglobin was 11.3g/dl with normal indices and differential white cell count and the ESR was 5mm/h. The basal metabolic rate determined by spirometry was low at 83.9kJ/h/m² (−45% of mean for age and sex). He was started on triiodothyronine replacement therapy and the dose titrated to his TSH response. He improved rapidly both symptomatically and physically. Within three months, ECG, EMG and nerve conduction tests reverted to normal and he reported that he had not felt so well for at least a year. BMR rose to normal (−4%, 153kJ/h/m²).

METHODS

Serum total T_4, total T_3 and TSH were measured by radioimmunoassay using polyethyleneglycol-accelerated double antibody methods and antisera developed in this laboratory. Serum total T_4 was also measured following extraction of serum by an acid-ethanol method.[1] Serum 'free' T_4 was measured by equilibrium radioimmunoassay using a commercial kit (IM 2050) obtained from Amersham International. Further free T_4 measurements were made by equilibrium dialysis[2] and by a two-step solid-phase antibody method.[3]

Endogenous thyroid hormone antibodies:

(i) Increasing (five-fold) dilutions of serum (50μl) were incubated with 200μl aliquots of either iodinated T_4 (800pg), T_3 (10pg) or the Amersham T_4 analogue (an unknown amount). After 4 hours incubation at room temperature, aliquots (250μl) of normal carrier serum were added to the mixture followed by an equal volume (500μl) of a 20% solution of PEG 6000. After thorough mixing, the immunoglobulin fraction was precipitated by centrifugation and the associated radioactivity counted. Binding of the tracers by the patient's serum was compared with that to normal serum specimens.

(ii) The patient's serum was used at a 1:25 final dilution to set up a thyroxine immunoassay standard curve using iodinated-T_4 as tracer and a variable amount of unlabelled T_4 within the range 0 − 480nmol/l. After overnight incubation at 4°C, bound and free labelled thyroxine were separated by the addition of carrier serum and PEG as described above. Data was computed by Scatchard analysis. Cross reactivity was estimated by a similar method and by comparing the percentage of bound tracer in the presence of a range of concentrations of unlabelled T_4 (0 − 480nmol/l) or thyroglobulin (0 − 230nmol/l).

(iii) An aliquot (100μl) of the patient's serum was incubated with 4ng of T_4 tracer for 2 hours at room temperature. A sample (10μl) of the incubate was subjected to separation by electrophoresis on cellulose acetate. Aliquots of normal serum treated similarly were run in parallel. A marker strip was stained with Ponceau S and the sample strips were sliced to correspond with the marker bands. The slices were counted for radioactivity content.

(iv) Serum was examined for T_4 autoantibodies by an ELISA system. The method uses T_4-coated micro-titred plates and enzyme-labelled antihuman IgG antibodies as the detector.[4]

Thyroid microsomal and thyroglobulin autoantibodies were estimated using commercial haemagglutination kits (Wellcome Reagents Ltd, London).

TABLE

Thyroid hormone profile at presentation

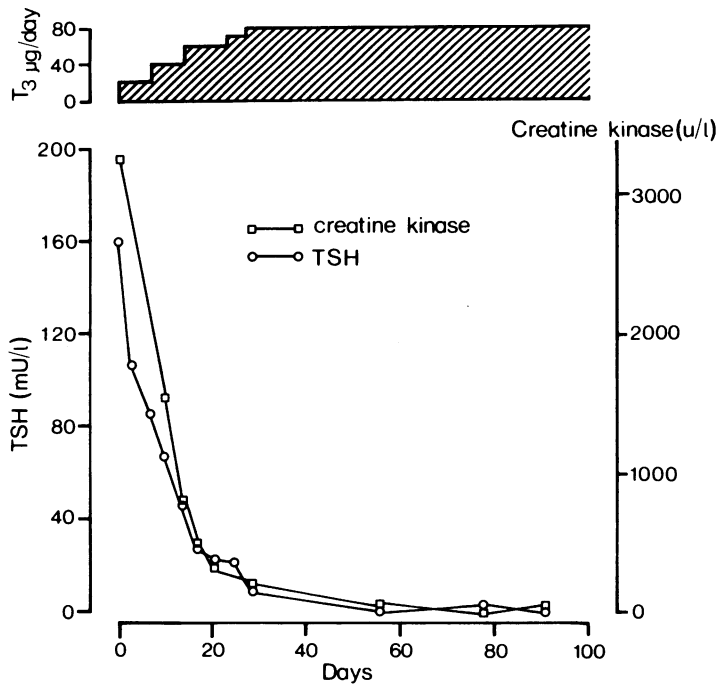
	Result	*Reference range*
Total T_4 (direct)	216	60 – 160 nmol/l
Total T_4 (extracted)	< 20	60 – 160 nmol/l
Total T_3	0.5	1.2 – 3.0 nmol/l
Free T_4 (Amerlex)	163	9 – 28 pmol/l
Free T_4 (2-step)	1	—
Free T_4 (dialysis)	5	9 – 17 pmol/l
TSH—basal	160	< 5 mU/l
—20 min post TRH (200 µg)	205	> 10 < 40 mU/l
—60 min post TRH	230	> 10 < 40 mU/l
TBG	9.7	7 – 17 mg/l
Anti-thyroglobulin Ab	1:320	
Anti-thyroid microsomal Ab	1:10000	

RESULTS

Thyroid function tests on presentation are shown in the Table. The serum total T_4 by the 'in house' radioimmunoassay was moderately elevated and the serum 'free' T_4 by the widely used Amerlex radioimmunoassay was very considerably elevated into the range for severe hyperthyroidism. Nevertheless the basal serum TSH was very high, consistent with primary hypothyroidism. The reason for the conflict in the results is suggested by the low serum total T_3, in the hypothyroid range. The true low values for total T_4 and free T_4 by methods which eliminated interfering factors ultimately confirmed that the patient had primary hypothyroidism. The effect of treatment with triiodothyronine on serum TSH and creatine kinase activity is shown in the Figure opposite.

The patient's serum bound 75.0% of the ^{125}I-T_4 tracer compared with only 4.1% binding by a control serum. It also bound 66.5% of the ^{125}I-T_4 analogue compared with 7.5% binding by control serum. Iodinated T_3 was bound 13.0% by the patient's serum and 11.2% by control serum. These tests were repeated many times but the results remained essentially unchanged throughout the first 100 days of treatment. After electrophoresis 72.2% of the T_4 tracer activity recovered was associated with the γ-globulin fraction of the patient's serum and 13.9% and 10.4% with the TBG and albumin/pre-albumin fractions respectively. The control serum produced values of 1.0% (γ-globulin), 81.2% (TBG) and 15.2% (albumin/pre-albumin). A positive reaction in the ELISA test confirmed the presence of T_4-binding IgG.

The effect of treatment with triiodothyronine on TSH & creatine kinase activity



The association constant of the autoantibodies for T_4 at 4°C calculated by Scatchard analysis was 1.2×10^8 litres/mole and the concentration of binding sites 0.3umol/l. Cross reactivity of the autoantibodies with thyroglobulin was between 200% and 900% of that with thyroxine on a molar basis, the exact figure depending on the incubation conditions. Thyroid microsomal and thyroglobulin autoantibodies were present in titres of 1:10,000 and 1:320 respectively on presentation and had fallen to 1:640 and 1:160 respectively after three months treatment.

DISCUSSION

Thyroid hormone-binding proteins in the γ-globulin region of the electrophoretogram were first described in a patient with papillary carcinoma of the thyroid following radioiodine treatment.[5] They have subsequently been described in euthyroid subjects, patients with autoimmune thyroiditis, Graves' disease, nontoxic nodular goitre and in hypothyroid patients treated with dessicated thyroid extract[6-10] and, rarely, in nonthyroidal illness. The subject has recently been reviewed.[11] The prevalence of thyroid hormone-binding in thyroid screening studies has been variously described as lying within the region 0.05%[12] to 1%.[13] The antibodies are directed against T_3 or T_4 though sometimes both occur in the same patient.[7, 9]

The anomalous thyroid function tests in the present case raised our suspicion of autoantibodies directed against one or both of the thyroid hormones. Laboratory analyses confirmed the presence of IgG antibodies capable of binding T_4 to a degree which was sufficient to explain the spurious results. The double antibody

separation method of RIA used in our laboratory led to a falsely high total T_4. Acid-ethanol extraction of the serum to eliminate interfering factors from the assay system revealed a true serum total T_4 concentration below the lowest standard (<20nmol/l). Similar interference was observed with the thyroxine analogue kit (Amerlex) which purports to measure 'free' T_4. Measurement of free T_4 by such analogue methods has been the subject of much ongoing controversy as to the soundness of the methodological precepts.[14, 15] Measurement of free T_4 by methods not susceptible to the presence of abnormal binding proteins revealed very low levels. The diagnosis of primary hypothyroidism was confirmed by the high TSH and low total T_3 levels, the response to TRH and the clinical improvement produced by treatment with triiodothyronine.

The calculated association constant of the T_4 autoantibody was low compared with other published estimates[7, 8, 9, 16] and its binding capacity was roughly equivalent to that of TBG. Consequently interference in the T_4 assay was relatively slight due to only partial sequestration of the iodinated T_4 used as tracer in the assay. This resulted in an apparently normal or only mildly elevated total T_4 level thereby allaying suspicion that the analytical result was very wrong and putting the clinical diagnosis in doubt. Antibodies of greater avidity and/or titre would have bound virtually all the assay label recording a result of such proportions that suspicion of its validity would have been raised immediately. In other cases[9, 17] the binding characteristics of the antithyroid hormone antibodies were such as to suggest a definitive role for them in the development of the hypothyroid state.

Investigation of the autoantibody in the present case confirmed thyroglobulin as a more specific antigen than T_4. There is a marked correlation between the presence of antithyroglobulin antibodies and antithyroid hormone antibodies[8] often with preferential binding of thyroglobulin to the antithyroid hormone antibodies[16, 18] suggesting a more extensive recognition site. The absence of (a significant titre of) antithyroglobulin antibody has, however, also been recorded[6, 18] but this may be a methodological artefact due to the presence of haemagglutination-inhibiting factors which can occur in autoimmune thyroid disease.[19] The fact that the autoantibodies showed minimal binding to T_3 suggested replacement therapy with triiodothyronine as the treatment of choice. The results of treatment on TSH and creatine kinase activity reveal their sensitivity to even suboptimal doses of T_3. This patient's lymphocytes have now been fused with a mouse myeloma cell line (Dr K Tan, Department of Biochemistry, University of Surrey, Guildford — personal communication) and the resulting hybridomas are producing thyroxine binding antibodies.

This case illustrates the need for further investigation in a patient whose symptoms and signs do not match laboratory results and emphasises TSH measurement as the investigation of choice for suspected primary hypotyhroidism.

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REFERENCES

1. Daughaday WL, Mariz IK, Blethen SL. Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radio-receptor and radioimmunoassay of somatomedin in native and acid-ethanol extracted serum. *J Clin Endocrinol Metab* 1980; **51**: 781-8.
2. Gow SM, Kellet HA, Toft AD, Beckett GJ. Accuracy and precision of five analog radioimmunoassays for free thyroxin compared. *Clin Chem* 1985; **31**: 1888-92.
3. Ekins RP. The direct immunoassay of free (non-protein bound) hormones in body fluids. In: Hunter WM, Corrie JET, eds. Immunoassays for clinical chemistry. Edinburgh: Churchill-Livingstone, 1983; 319-39.
4. Goodburn R. Biochemical and clinical investigations of thyroid autoantibody antigen systems *in vitro* using labelled antibody techniques. Guildford: University of Surrey, 1983. PhD thesis.
5. Robbins J, Rall JE, Rowson RW. An unusual instance of thyroxine-binding by human serum gammaglobulin. *J Clin Endocrinol Metab* 1956; **16**: 573-9.
6. Wu S-Y, Green WL. Triiodothyronine (T_3)-binding immunoglobulins in a euthyroid woman: effects on measurement of T_3 (RIA) and on T_3 turnover. *J Clin Endocrinol Metab* 1976; **42**: 642-52.
7. Ginsberg J, Segal D, Ehrlich RM, Walfish PG. Inappropriate triiodothyronine (T_3) and thyroxine (T_4) radioimmunoassay levels secondary to circulating thyroid hormone autoantibodies. *Clin Endocrinol* 1978; **8**: 133-9.
8. Staeheli V, Valloton MB, Burger A. Detection of human antithyroxine and anti-triiodothyronine antibodies in different thyroid conditions. *J Clin Endocrinol Metab* 1975; **41**: 669-75.
9. Beckett GJ, Todd JA, Hughes GJ, Campbell IW. Primary hypothyroidism with grossly elevated plasma total thyroxine and triiodothyronine levels. *Clin Endocrinol* 1983; **19**: 295-303.
10. Beck-Peccoz P, Romelli PB, Cattaneo MG, et al. Evaluation of free thyroxine methods in the presence of iodothyronine-binding autoantibodies. *J Clin Endocrinol Metab* 1984; **58**: 736-9.
11. Sakata S, Nakamura S, Miura K. Autoantibodies against thyroid hormones or iodothyronine. *Ann Int Med* 1985; **103**: 579-89.
12. Fielding AM. Prevalence of serum autoantibody binding of Amerlex thyroxin analog. *Clin Chem* 1984; **30**: 501-2.
13. Mullinger RN, Walker G. Free thyroxin in thyroid disease. *Clin Chem* 1982; **28**: 1394-5.
14. Ekins R, Jackson T. Letter. *Lancet* 1985; **2**: 782.
15. Wilkins TA, Midgley JEM, Barron N. Comprehensive study of a thyroxin-analog-based assay for free thyroxin ('Amerlex FT4'). *Clin Chem* 1985; **31**: 1644-53.
16. Konishi J, Iida Y, Kousaka T, Ikekubo K, Nakagawa T, Torizuka K. Effect of anti-thyroxin autoantibodies on radioimmunoassay of free thyroxin in serum. *Clin Chem* 1982; **28**: 1389-91.
17. Karlsson FA, Wibell L, Wide L. Hypothyroidism due to thyroid-hormone binding antibodies. *N Engl J Med* 1977; **296**: 1146-8.
18. Pearce CJ, Byfield PGH, Edmonds CJ, Lalloz MRA, Himsworth RL. Autoantibodies to thyroglobulin cross-reacting with iodothyronines. *Clin Endocrinol* 1981; **15**: 1-10.
19. Wilkin TJ, Swanson Beck J, Hayes PC, Potts RC, Young RJ. A passive haemagglutination (TRC) inhibitor in thyrotoxic serum. *Clin Endocrinol* 1979; **10**: 507-14.