Case report

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

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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 (T₄) levels which were initially misleading. Further investigations revealed the presence of IgG autoantibodies to T₄.

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 T₄ 124nmol/l (reference range 60 – 160), serum 'free' T₄ 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.
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. Serum 'free' $T_4$ was measured by equilibrium radioimmunoassay using a commercial kit (IM2050) obtained from Amersham International. Further free $T_4$ measurements were made by equilibrium dialysis and by a two-step solid-phase antibody method.

*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.

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(iv) Serum was examined for T4 autoantibodies by an ELISA system. The method uses T4-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 T4 (direct)| 216    | 60 - 160 nmol/l   |
| Total T4 (extracted) | < 20  | 60 - 160 nmol/l   |
| Total T3         | 0.5    | 1.2 - 3.0 nmol/l  |
| Free T4 (Amerlex)| 163    | 9 - 28 pmol/l     |
| Free T4 (2-step) | 1      | —                 |
| Free T4 (dialysis)| 5    | 9 - 17 pmol/l     |
| TSH—basal        | 160    | < 5 mU/l          |
| —20 min post TRH (200μg) | 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 T4 by the 'in house' radioimmunoassay was moderately elevated and the serum 'free' T4 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 T3, in the hypothyroid range. The true low values for total T4 and free T4 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 125I-T4 tracer compared with only 4.1% binding by a control serum. It also bound 66.5% of the 125I-T4 analogue compared with 7.5% binding by control serum. Iodinated T3 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 T4 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 T4-binding IgG.
Thyroxine-binding autoantibodies

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

![Graph showing TSH and creatine kinase activity over time.]

The association constant of the autoantibodies for T₄ at 4°C calculated by Scatchard analysis was 1.2 × 10⁸ litres/mole and the concentration of binding sites 0.3 μmol/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 electrophoreto-gram were first described in a patient with papillary carcinoma of the thyroid following radiiodine treatment. 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 and, rarely, in nonthyroidal illness. The subject has recently been reviewed. The prevalence of thyroid hormone-binding in thyroid screening studies has been variously described as lying within the region 0.05% to 1%. The antibodies are directed against T₃ or T₄ though sometimes both occur in the same patient.

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 T₄ 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 T₄. Acid-ethanol extraction of the serum to eliminate interfering factors from the assay system revealed a true serum total T₄ concentration below the lowest standard (<20 nmol/l). Similar interference was observed with the thyroxine analogue kit (Amerlex) which purports to measure 'free' T₄. Measurement of free T₄ by such analogue methods has been the subject of much ongoing controversy as to the soundness of the methodological precepts. Measurement of free T₄ 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 T₃ levels, the response to TRH and the clinical improvement produced by treatment with triiodothyronine.

The calculated association constant of the T₄ autoantibody was low compared with other published estimates and its binding capacity was roughly equivalent to that of TBG. Consequently interference in the T₄ assay was relatively slight due to only partial sequestration of the iodinated T₄ used as tracer in the assay. This resulted in an apparently normal or only mildly elevated total T₄ 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 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 T₄. There is a marked correlation between the presence of antithyroglobulin antibodies and antithyroid hormone antibodies often with preferential binding of thyroglobulin to the antithyroid hormone antibodies suggesting a more extensive recognition site. The absence of (a significant titre of) antithyroglobulin antibody has, however, also been recorded but this may be a methodological artefact due to the presence of haemagglutination-inhibiting factors which can occur in autoimmune thyroid disease. The fact that the autoantibodies showed minimal binding to T₃ 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 T₃. 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 hypothyroidism.

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