Euthyroid hyperthyroxinaemia due to assay interference

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Abstract

Background: The authors report a case of euthyroid hyperthyroxinaemia and the systematic approach that led to the diagnosis. The related literature is also reviewed in an attempt to increase awareness of this condition.

Case report: A 47-year-old female patient was referred for further investigation and management of "hyperthyroidism." The patient was clinically euthyroid and had previously been treated with carbimazole, but self-discontinued therapy as she felt unwell on treatment. A careful review of this patient's blood results revealed elevated free thyroxine and unsuppressed thyroid-stimulating hormone (TSH). This is atypical of primary hyperthyroidism, in which case suppressed TSH would have been expected. In view of the clinical euthyroidism, euthyroid hyperthyroxinaemia was considered the most likely diagnosis and an appropriate work-up was initiated. Following on the consultation with the Chemical Pathology Unit, assay interference was established as the likely cause and the patient was reassured. She remains well, with no treatment.

Conclusion: Thyroid function tests should not be interpreted in isolation and, if the clinical picture and biochemistry are discordant, it is imperative to consider assay interference. It is also important to apply basic physiological principles in interpreting endocrine blood results. In this patient, both the clinical euthyroidism and the unsuppressed TSH, which are atypical of primary hyperthyroidism, prompted further work-up.

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Introduction

Thyroid dysfunction is often invoked as a cause for numerous non-specific and nebulous symptoms in the absence of supporting clinical findings. In other instances, thyroid function tests may be performed as a routine in an asymptomatic patient. Abnormal results may be obtained, resulting in an erroneous diagnosis. The presence of euthyroid hyperthyroxinaemia in these circumstances may result in an erroneous diagnosis of hyperthyroidism. An unusual cause of euthyroid hyperthyroxinaemia is described.

Case report

A 47-year-old female patient presented to her gynaecologist in December 2008 for a routine check-up. She had no symptoms relating to her thyroid and reported no loss of weight, no nervousness, no palpitations, and no ocular symptoms. In fact, she generally felt very well. She had a family history of thyroid dysfunction in that her mother had hypothyroidism and was taking L-thyroxine. This prompted a routine thyroid function test to be performed by the gynaecologist. Her thyroid function test was reported to indicate hyperthyroidism, but with unsuppressed thyroid-stimulating hormone (TSH) despite elevated thyroxine (T_4; Table I). The patient elected to commence with no treatment, as she was going on holiday. The patient was seen by her gynaecologist again in January 2009, during which time her thyroid function test again showed elevated T_4 with unsuppressed TSH. The patient was again completely asymptomatic. Based on the isolated elevated T_4 result, she was commenced on carbimazole 10 mg daily, but discontinued her therapy shortly thereafter as she felt very unwell on treatment. She revisited the doctor again in June 2009 and, following repeat thyroid function testing that yet again indicated the elevated T_4 and unsuppressed TSH, the patient was referred to our centre.

Clinically, the patient was euthyroid and normal in all respects. In view of the clinical findings, a possibility of

Table I: Results of serial thyroid function tests

	Elecsys® 2010 (Roche)				ARCHITECT® i8200 (Abbott)	
Date	**December 2008**	**January 2009**	**June 2009**	**Normal range**	**July 2009**	**Normal range**
TSH (µIU/ml)	0.39	0.52	0.48	0.27–4.20	0.68	0.35–4.94
Free T_3 (pmol/l)	-	-	12.4	2.8–7.1		
Free T_4 (pmol/l)	44.4	33.7	32.5	12.0–22.0	13.1	9.0–19.0

assay interference was considered to be the most likely explanation for the lack of clinical correlation with the blood results. Thyroid hormone resistance and TSH-producing tumour was also considered, although deemed less likely.

Blood samples were submitted to the Chemical Pathology Unit at Lancet Laboratories in Johannesburg, South Africa and analysed in three separate assays. Two showed a completely normal result, in keeping with euthyroidism, and one showed an elevated T_4 and triiodothyronine (T_3) in the face of unsuppressed TSH (Table I). The Roche Elecsys® assay that was showing the abnormal result was analysed in more detail, and an interfering factor to the ruthenium label was identified in the sample. More detailed analysis revealed that all of the previous blood tests that were shown to be abnormal had been performed with the Roche assay.

The patient was reassured and it was recommended to her that in the future she should have her blood tests done on the assays that showed no interference to avoid confusion and unnecessary investigations, treatment and anxiety.

Discussion

The term "euthyroid hyperthyroxinaemia" may be used to describe any condition in which serum T_4 is increased in the absence of thyrotoxicosis.[1] Despite a number of publications on the subject, conditions causing euthyroid hyperthyroxinaemia are frequently unrecognised, and the discrepancy between a patient's clinical state and test results is overlooked.[2] Much of the literature on euthyroid hyperthyroxinaemia is from the 1980s and there have been few publications on the topic since then.

Familial dysalbuminaemic hyperthyroxinaemia (FDH) is due to albumin with an abnormal binding site that shows much greater affinity for thyroxine than the hormone-binding site on thyroxine-binding globulin (TBG).[3] FDH is an autosomal dominant disorder that results in increased total T_4, but free T_4 and total and free T_3 remain normal in the otherwise euthyroid patient.[4] This patient had increased free T_4 and elevated free T_3, and no family history of a similar problem, making the diagnosis unlikely.

Causes such as psychiatric illness and drugs could be largely excluded by taking the clinical context into account.[5] It is important to interpret thyroid function tests with care in acute psychiatric admissions.[6] Resistance to thyroid hormone (RTH) is uncommon and is characterised by reduced responsiveness of the target tissues to circulating thyroid hormones. The biochemical hallmark is elevated free T_4 and non-suppressed pituitary TSH, reflecting resistance to thyroid hormone action in the hypothalamic-pituitary-thyroid axis.[5] This patient had no goitre (present in up to 65% of individuals with RTH) and no symptoms relating to her cardiovascular, musculoskeletal or central nervous systems, and also no hearing loss or abnormal colour vision. Serum immunoglobulins, which may be reduced in individuals with RTH, were normal.[5] The patient also had documented antibody interference and normal free T_4 and TSH on a two-step assay, thus making the diagnosis of RTH unlikely. Factitious hyperthyroxinaemia has been described due to immunoglobulin (Ig) A-secreting multiple myeloma.[7] This patient's serum protein electrophoresis was entirely normal.

TSH-secreting pituitary adenoma was excluded by normal sex hormone-binding globulin, a normal magnetic resonance imaging scan (done for other indications) of the pituitary and with normal thyroid function tests on two-step assay, and thus other investigations (thyrotropin-releasing hormone response, T_3 suppression, α-subunit:TSH ratio) were not considered necessary.

This patient had euthyroid hyperthyroxinaemia on the basis of antibody interference and we will discuss this in more detail.

An evaluation of the patient's laboratory records showed that two methods from different manufacturers using different principles were used at different times to determine her thyroid hormone levels. The ARCHITECT® method (Abbott Laboratories) consistently produced results concordant with the clinical picture, whereas Elecsys® (Roche Diagnostics) appeared to produce results that were discordant with the clinical picture in this patient. The ARCHITECT® method for free T_4 (FT_4) and free T_3 (FT_3) is a two-step assay in which

patient serum and labelled FT_4/FT_3 are separated by a wash step. This reduces the sequestration of labelled FT_4/FT_3 through nonspecific binding.

Principle of the FT_4/FT_3 Roche assay

The FT_4/FT_3 assay uses an electrochemiluminiscent competitive binding immunoassay. This allows results to be available in a very short time to allow better patient management. The assay is known as a one-step, two incubations method.

First incubation: The patient sample is mixed with specific sheep-derived polyclonal antibodies for FT_4, or, in the case of FT_3, with sheep-derived monoclonal antibodies. These antibodies are labelled with ruthenium complex [ruthenium (II) tris (bipyridyl) complex].

Second incubation: There is no wash step. The reagent mixture containing biotinylated T_4 (or T_3) and streptavidin-coated paramagnetic microparticles is added. Biotinylated T_4/T_3 binds to any free binding sites of the specific polyclonal T_4 or monoclonal T_3 antibodies. Streptavidin and biotin react to form an antibody-hapten complex. The more free binding sites are available, the more labelled hormone will bind to these sites. The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the electrode. All unbound substances, including serum FT_4-ruthenium-labelled specific antibody complex, are washed out with Procell®.

The application of a voltage to the electrode induces chemiluminiscent emission, which is measured. The emission of a chemiluminiscent signal is inversely proportional to the hormone present in the patient serum.[8]

When clinically discrepant results were observed in this patient, further investigations that were conducted included re-analysis of the same sample by two different manufacturers' methods, rheumatoid factor (RF), thyroid autoantibodies, as well as the shipping of sample aliquots to Roche for further investigations.

The two manufacturers' products yielded discrepant results (Table II). The patient was negative for RF and thyroid autoantibodies. According to the manufacturer, heterophile antibodies (IgG and IgM type) were identified as interfering proteins. These antibodies most likely block the ruthenium-labelled specific T_4 and T_3 antibodies from binding to biotinylated T_4/T_3, thus causing a signal quench that, in turn, results in high FT_4/FT_3 results.

Polyethylene glycol (PEG) precipitation or dilution studies were not carried out on these samples.

Table II: Same sample tested by two methods

Date	Method/platform	TSH (µIU/mL)	FT_4 (pmol/L)
21 August 2009	ARCHITECT® i8200	0.85	15.3
21 August 2009	Elecsys® 2010	0.35	30.7

Antibody interference in thyroid assays may result in apparent abnormal concentrations of thyroid hormones inconsistent with the patient's thyroid state.[8] Thyroid hormone can be measured with single- or double-antibody immunoassays.[9] When interpreting thyroid function tests it is important to take into account basic physiology, as this will allow for internally inconsistent results to be appreciated. In primary hyperthyroidism, TSH becomes suppressed prior to FT_4 and FT_3 elevation, in an attempt to maintain euthyroidism. The presence of elevated FT_4 in the presence of TSH that is inappropriately non-suppressed, as was the case with this patient, would not be in keeping with primary hyperthyroidism.

The presence of circulating, endogenous antibodies directed against a number of antigens may cause both falsely depressed and falsely increased values in thyroid hormones. The outcome largely depends on the nature of the interfering antibody or the assay design. The major importance of appreciating antibody interference as a confounding factor in the interpretation of thyroid function tests is that it often leads to inappropriate investigations and treatment, as was the case in this patient.

In thyroid hormone immunoassays, the major sources of antibody interference are autoantibodies, heterophile antibodies and RF. Autoantibodies as interfering factors include antibodies to thyroglobulin, microsomal thyroid peroxidase and TSH receptor, and antibodies reacting with T_4 and T_3. Many different approaches may be utilised to overcome the interference, e.g. PEG precipitation. Heterophile antibodies are known to interfere with many immunoassays, and are antibodies against specific animal immunoglobulins or against immunoglobulins of various animal species. The best-known heterophile antibodies are human anti-mouse antibodies. RF may also exhibit non-specific binding to the analytical antibodies and cause interference. The non-specific binding by RF may be overcome, as for heterophile antibodies, with blocking reagents such as non-immune homologous immunoglobulin.[10]

When interpreting a thyroid function test, it is important to consider antibody interference in a patient with discrepant

results. Routine communication between the chemical pathologist and the clinician is imperative to delineate further the nature of the problem. This will allow for a discrepancy between the clinical findings and the laboratory findings to be followed up, as was the case with this patient. The laboratory should then repeat the suspect sample to confirm if the interfering antibodies could account for the spurious result. Samples are typically re-evaluated using an alternative method and the removal of the interfering antibody (e.g. by PEG), or by using antibody-blocking reagents. Results on reanalysis that are different after the removal of interfering antibodies are indicative of antibody interference. These results are not reportable, as they may not reflect realistic concentrations.

Conclusion

Both chemical pathologists and clinicians must be vigilant to the possibility of antibody interference when interpreting thyroid function tests, particularly with the finding of euthyroid hyperthyroxinaemia. This will prevent unnecessary investigations and treatment. This case represents an unusual but important problem in clinical endocrinology.

References

1. Farror C, Wellby ML, Beng C. Familial dysalbuminaemic hyperthyroxinaemia and other causes of euthyroid hyperthyroxinaemia. J R Soc Med. 1987;80:750–752.
2. Rippere V. Euthyroid hyperthyroxinaemia. J R Soc Med. 1988;81:369.
3. Ruiz M, Rajatanavin R, Young RA, et al. Familial dysalbuminemic hyperthyroxinemia: a syndrome that can be confused with thyrotoxicosis. N Engl J Med. 1982;306:635–639.
4. Kronenberg HM, Melmed S, Polonsky KS, Larsen PR. William's textbook of endocrinology. Philadelphia: Elsevier, 2008; p. 306.
5. Resistance to thyroid hormone. In: DeGroot LJ, Jameson JL, editors. Endocrinology. 5th edition. Philadelphia: Elsevier, 2006; p. 2227–2237.
6. Lambert TJ, Davidson R, McLellan GH. Euthyroid hyperthyroxinaemia in acute psychiatric admissions. Aust N Z J Psychiatry. 1987;21:608–612.
7. Cissewski K, Faix JD, Reinwein D, Moses AC. Factitious hyperthyroxinaemia due to monoclonal IgA in a case of multiple myeloma. Clin Chem. 1993;39:1739–1742.
8. Weir DM. Elecsys® 2010. Operator's manual. Indianapolis: Roche Diagnostics; 2005.
9. Zouwail SA, O'Toole AM, Clark PMS, Begley JP. Influence of thyroid hormone autoantibodies on 7 thyroid hormone assays. Clin Chem. 2008;54:927–928.
10. Despres N, Grant AM. Antibody interference in thyroid assays: a potential for clinical misinformation. Clin Chem. 1998;44:440–454.