INTERFERENCE OF ANTI-T3 AUTOANTIBODIES IN THE MEASUREMENT OF TOTAL AND FREE T3 IN SERUM

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ABSTRACT

A case of anti-triiodothyronine autoantibodies is presented in this report. The thyroid hormone profile of a hypertensive patient, with multinodular goiter and history of exogenous thyroid hormone therapy, was found to be highly ambiguous. The total as well as free T3 levels were consistently high (out of range) whereas the T4 (total and free) values were always within normal limits. The thyrotropin was found to be partially suppressed. Very low T3-Uptake indicated some kind of interference in the immunoassays. We incubated the sera with the radio-iodine labelled T3 and observed that the patient's serum bound about ten times more radioactivity than a control run in parallel. On further resolving the serum proteins on cellulose acetate electrophoresis, the radioactivity was detected in the γ -globulin band. Therefore it was established that the patient's serum carried the antibodies reactive with T3 which were interfering in the immunoassays. Elevated anti-thyroid microsomal antibodies were also present in the patient's serum. The anti-T3 antibodies were highly specific for T3 and did not show any cross reactivity with the T4 or its analogues used in the estimation of free T4.

KEY WORDS: Thyroid antibodies, anti-T3 antibodies, T3 RIA

It is well established that circulating antibodies to various thyroid antigens like thyroperoxidase, thyroglobulin, thyrotropin receptors and microsomal proteins may be found in various autoimmune thyroid disorders (1). It is very rare that such autoantibodies are generated against the thyroid hormones themselves (2,3). The presence of these antibodies in serum does not only complicate the clinical manifestations of the disease but also interferes with the estimation of total and free hormones by immuno assay (3,4,5). Their presence may be indicated when very low (undetectable) values of either or both T3 and T4 are observed in a competitive RIA/EIA that uses precipitation for the separation of bound and free ligand (6). However, in immunoassays using antibody coated tubes extremely high values are

observed (7). Sakata et al (1994) screened 880 apparently healthy adults and reported that the incidence of anti-T3 or anti-T4 antibodies was 1.8% (4). The minimal effect of the antibodies on assays in these individuals could apparently be due to the lower association constants of these antibodies as compared to those used in the immunoassays. The antibodies in most of the cases are specific for either of the two hormones although concomitant occurrence of anti-thyroperoxidase or anti-microsomal antibodies has been reported (7,8).

Case Report: A 50 year old hypertensive female with multinodular goiter and history of palpitations was referred to our laboratory for thyroid function evaluations. She had been put on levo-thyronine for more than ten years for hypothyroidism diagnosed by low radio-iodine uptake. The patient had consistent menorrhagia along with occasional palpitations during all these years. Her thyroid hormone profile

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showed exceedingly high total T3 levels (out of range) whereas T4 and FTI were normal or on the higher side of normal range.

In view of the very high T3 levels and occasional palpitations the patient was diagnosed as a case of T3-toxicosis and put on neomercazole.

The menorrhagia and palpitations were controlled in about three months, although the thyroid hormone profile remained unchanged. The unconformity of high T3 levels with improvement in the clinical condition remained an unanswered question.

We investigated the case in our laboratory, suspecting the presence of some interfering agent in the patient's serum in the form of anti-T3 autoantibodies or high affinity albumin (familial dysalbuminemia).

MATERIALS AND METHODS

Assay of Hormones: Total T3, T4 and thyrotropin concentrations as well as the percent T3-uptake were assayed in unextracted serum by RIA using the commercial kits supplied by Diagnostic Products Corporation, USA. These kits were polyclonal anti-T3 (or T4) antibodies coated on the polypropylene tubes and are based on competitive type of RIA. The titre of anti-thyroid microsomal antibodies was measured with the help of an EIA kit from Clark Inc., USA.

Demonstration of Anti-T3 antibodies

a) **Binding of the Tracer:** The method described by Allan et al (9) was used with some modifications to detect the binder in the patient's serum. The serum along with a control serum (a healthy subject with normal thyroid hormone profile) was incubated with radioactive T3, T4, T3-analogue, T4-analogue from the commercial kits mentioned above. The incubation was carried out for 2 hours

in uncoated glass tubes followed by precipitation with 7.5% polyethylene glycol (PEG). The precipitates were separated by centrifugation at 1500 x g for 15 minutes followed by a washing with 7.5% PEG.

b) **Fractionation of the binder by electrophoresis:** The patient's serum, as well as the control serum proteins were fractionated on cellulose acetate strips by electrophoresis. After visualization with the help of Poncaeu-S stain, the strips were scanned on a densitometer (Beckman, USA). The protein bands, were cut and incubated overnight at room temperature with the radiolabelled (^{125}I) T3 in the presence as well as absence of 8-anilino-1-naphthaleno-sulphonic acid (ANS). The excess of the tracer was removed by two washings with 0.5% tween-20. The radioactivity bound with the protein bands was counted in a multicrystal γ counter (Berthold, W. Germany).

RESULTS AND DISCUSSION

Thyroid Profile: The thyroid function profile of the patient is shown in Table 1. The thyroxine, free thyroxine estimate (FTI) as well as free-thyroxine were within normal limits although thyroxine levels were found to be slightly on the higher side of the normal range. The T3-uptake (by a kit that uses antibody coated tubes as binder) was found to be consistently lower. In Nov. 1995, it was highly suppressed and was responsible for lowering the FTI slightly below the normal range. Thyrotropin levels in the patient's serum were observed to be fluctuating below the normal range and were suggestive of slight suppression of the pituitary. We were not able to measure the concentration of triiodothyronine (T3) except for once i.e. in Nov. 1993. The same was true for the estimation of Free-T3. The patient's serum was also found to be positive for anti-thyroid-microsomal antibodies in Nov. 1995. A number of preanalytical and analytical factors may result in the aberrations in thyroid function testing (5) and the anomalies in the present case appeared to be because of the presence of an inherent T3-binder in the serum.

Table 1: Sequential laboratory findings (Thyroid profile) of the patient.

Investigation	Nov-1993	Sep-1994	Nov-1995	Normal
Total T3	16.6	>24.0	>24.0 ng/ml	(0.9-2.0)
Total T4	9.4	11.0	10.3 µg/dl	(4.5-12.0)
Thyrotropin	0.08	0.25	0.22 uU/ml	(0.2-5.0)
T3-uptake	16.9	16.5	8.4%	(25.0-37.0)
FTI*	1.6	1.7	0.86 U	(1.0-4.5)
Free T3	-	-	>40.0 pg/ml	(2.5-5.8)
Free T4	-	-	2.4 ng/dl	(0.8-2.5)
Anti-Thyroid-Ms-Ab	-	-	45.0 IU/ml	(0.0-20.0)

* FTI: Free Thyroxine Index

The present patient showed mild symptoms of hyperthyroidism (menorrhagia responsive to thyroid suppressive therapy) along with borderline thyroxine and mildly suppressed thyrotropin levels. The absence of obvious symptoms of hyperthyroidism or clearly elevated thyroxine levels were contradictory to the extremely high T3 values in the patient's serum. The possibility of T3-thyrotoxicosis was also ruled out when the T3 values consistently remained out of range even when estimated in ten times diluted serum and with or without the antithyroid treatment.

In our attempt to demonstrate the presence of a specific T3-binder in the patient's serum, we incubated the serum with the radiolabelled T3. The patient's serum exhibited very strong binding of the tracer (Table 2). The binding capacity of the serum was more than nine times higher than that observed in the control serum i.e. serum of a euthyroid healthy individual. The binding of the tracer was unaltered in the presence of ANS, a specific blocker of the thyroid binding globulin (TBG) (10). The T3 analogue used in the free T3 immunoassay kit also showed a similar affinity for the patient's serum. The increased

Table 2: Binding of the Radio-labeled thyroid hormones with the serum proteins.

Tracer Used	Control (C) cpm	Patient (P) cpm	P:C Ratio
¹²⁵ I-T3	1250	11765	9.41
¹²⁵ I-T3 + ANS	878	6752	7.69
¹²⁵ I-T3-Analogue	976	7150	7.33
¹²⁵ I-T4	1600	1551	0.97
¹²⁵ I-T4-Analogue	1124	1045	0.93

patient's serum for radiolabelled ¹²⁵I-T4 as well as the T4-analogue did not differ from that of the control serum. This observation confirmed that the T4 levels as determined by RIA represented the true thyroid status i.e. the patient was essentially euthyroid. The extremely high levels of serum T3 in this patient can be explained by the presence of some interfering agent which appeared to compete with the antibodies used in the radioimmunoassays (3,7). The suppressed T3 uptake values also corroborated the idea of a high affinity binder in the serum. The binder reacting with radiolabelled T3 resulting in its precipitation could have been any of the serum proteins. The increased

affinity/capacity of different plasma proteins particularly albumin has been reported as in familial dysalbuminemic hyperthyroidism (11). Immunoglobulins have also been found to interfere in immunoassays, particularly in patients with thyroid autoimmune disease (3,7). Most often such a condition results in mild to moderate elevation in the levels of the thyroid hormones due to increased binding capacity of plasma proteins (5). The borderline high thyroxine and mildly suppressed thyrotropin also indicated such a condition.

In order to identify the binder in patient's serum,

the proteins separated on cellulose acetate strips were incubated with radiolabelled T3. Maximum incorporation of radioactivity was observed in the γ globulin fraction, being more than twice in the patient's than in the control as shown in fig. 1. The albumin fraction of the two sera bound equal amounts of radioactivity thus excluding the possibility of dysalbuminemic hyperthyroidism.

It has been suggested that, in autoimmune thyroid diseases, the iodothyronines within the thyroglobulin molecule simulate the behaviour of a hapten bound to a carrier and thus elicit the immune response to generate the antibodies (5). In this patient, the antibodies were specific for T3 and did not appear to have any affinity for T4 or its analogue as shown in table 2. The specificity of the anti-T3 antibodies enabled us to even standardize a T3 radioimmunoassay where only four microlitre of the patient's serum was sufficient to bind 50% of the radiolabelled T3 (unpublished results), thus giving us a standard curve in the physiological range. Characterization of the association constant and the capacity of antibodies as well as the development of a T3 RIA based on

purified antibodies from the patient's serum is presently being carried out in our laboratory.

The above report demonstrates the occurrence of T3 antibodies and the associated clinical dilemma. The presence of such anti-T3 and/or anti-T4 antibodies, although a rare occurrence, may present with bizarre clinical symptoms and laboratory findings. The associated presence of anti-thyroid microsomal and/or anti-thyroperoxidase titres has been found to be almost a consistent phenomenon (1,2). The very low T3 uptake can be an indication of such a condition, but this test may soon be a thing of the past as it provides very little information on thyroid function otherwise (12). In the present case anti-T3 antibodies interfered only with the estimation of total and free T3 and did not produce any aberrations in the T4 RIA as reported by many workers (13,14).

The simple incubation of the patients serum with radiolabelled thyroid hormone followed by the precipitation of immune complexes with polyethylene glycol were found to be very effective in demonstrating the presence of these antibodies.

Fig. 1: Binding of ^{125}I -T3 tracer with protein bands separated on cellulose acetate paper electrophoresis strips.

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