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Experimental study on abnormal thyroid function in patients with Hashimoto's thyroiditis caused by interference of thyroid hormone autoantibodies

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Abstract

Introduction: Thyroid hormone autoantibody (THAAb) is one of the important factors affecting the measurement of thyroid function. By studying the results of a patient suffering with Hashimoto's thyroiditis, we fully communicated with the clinician, looked for reasons, **and achieved the purpose of restoring the truth and sorting out solutions [???].**

Material and methods: During routine examination using an ADVIA-Centaur XP system, we found that the test of a case was inconsistent with her clinical manifestations, with abnormal elevation of free thyroxine (FT4) that did not conform to the rule of the hypothalamic-pituitary-thyroid axis. Then, different platforms and demonstration of THAAbs with polyethylene glycol (PEG) precipitation were performed to eliminate the influence of THAAbs.

Results: The results showed that the thyroid function of the patient was consistent with the clinical manifestations and conformed to the law of the hypothalamic-

pituitary-thyroid axis using an Architect-i2000sr platform and Roche-Cobas-601 system. The content of FT4 was significantly reduced and lower than the normal reference range after the patients' serum was treated with PEG, which was in line with the clinical practice. The serum THAAb titre of the patient was nearly 100 times higher than that of the control group.

Conclusions: Once the thyroid function of the patients do not conform to their own laws and clinical manifestations [??], laboratory staff should consider the **interference [??]** of THAAbs. It is necessary to change the detection platform and retest the serum after PEG treatment while communicating with the clinicians, which is of great significance to provide a true and accurate result to clinicians and patients.

Key words: Hashimoto's thyroiditis; thyroid function; thyroid hormone autoantibody; free thyroxine 4

Introduction

Autoantibodies are one of the many **interferences [??]** in clinical practice [1], which are often present in patients with autoimmune thyroid diseases. They directly target different antigens and exhibit nonspecific binding to markers in immunoassays. Thyroid hormone autoantibodies (THAAbs) affect the authenticity of thyroid hormone test results, leading to misdiagnosis or inappropriate treatment, and with unpredictable **certain** medical risks. The frequency of THAAbs is 1.8% in the healthy people [2], while the incidence is as high as 30–40% in patients with autoimmune thyroid disease [3, 4]. It is necessary to increasing the awareness of this type of analytical problem if laboratories are to avoid reporting incorrect test results. Here, we studied a patient with a falsely elevated test result due to the presence of serum THAAbs. We attempted to identify a method to correctly evaluate the thyroid function status in THAAb-positive patients.

Material and methods

Patient

A 61-year-old woman presented with fatigue and oedema for 4 years. She took methimazole because of detecting elevated free thyroxine (FT4) and thyroid-stimulating hormone (TSH) levels in another hospital. The patient's main manifestations were fatigue, weakness, chills, oedema, and constipation. After repeated examination, except the abnormal T4 and pituitary thyrotropin, the results of our hospital were consistent with the previous tests.

Analytical methods

Thyroid function tests (TFTs) [TSH, free triiodothyronine (FT3), and FT4] and the serum concentrations of total T3 and total T4 were measured by radioimmunoassay (RIA), chemiluminescence immunoassay on an ADVIA Centaur XP system and Architect i2000sr platform, and electrochemiluminescence immunoassay on a Roche Cobas 601 system, respectively.

For the autoantibody binding test, radiolabelled T3 or T4 was added to the serum of a patient and the mixed serum of a normal subject. Subsequently, equal volumes of 25% polyethylene glycol (PEG) solution were added to the samples after incubated for 2 h at 37℃. Then, the mixture was centrifuged (4℃, 10,000 rpm) for 20 min, and the radioactivity of the precipitate was determined. The presence of antibodies against T3 or T4 was determined in accordance with the ratio of the count of radioactivity to the total radioactivity (B/T). The concentrations of T3, T4, and TSH in the PEG-treated supernatant were determined.

Determination of THAAb

The patients' serum samples and 20 normal subjects were added with radiolabelled T3 and T4 analogues. The titres of anti-T3 and -T4 autoantibodies were determined through RIA. Nonspecific binding was subtracted from all sample readings, and the final percentage of [^{125}I] T4 or [^{125}I] T3 binding was calculated. The result was expressed as the antigen-antibody binding rate.

Results

Comparison of thyroid function between different test platforms

The TFT of a patient diagnosed with Hashimoto's thyroiditis on the ADVIA Centaur XP system revealed increased serum FT4 and TSH, and reduced FT3. The results did not conform to the hypothalamic-pituitary-thyroid axis and the clinical manifestations. Then, we tested TT3 and TT4 to identify the reason for this discrepancy. Surprisingly, the results were consistent with the laws of the hypothalamic–pituitary–thyroid axis and the clinical manifestations. Subsequently, we detected FT3\FT4\TSH through RIA and with the Architect i2000sr platform and Roche Cobas 601system. T3 and T4 levels were simultaneously detected by using the Architect i2000sr platform and Roche Cobas 601 system. The results were in accordance with regular rules. As shown in **Table 1**, the FT3 and TSH results from the four different detection systems show consistent trends, and the FT4 results are clinically consistent with the results of most platforms, except for the Siemens detection system.

Results of autoantibody binding experiment and TFT before and after PEG treatment

Specific binding to I^{125}-T4 was observed in the patient’s serum. However, nonspecific binding to I^{125}-T4 was also found in the serum of normal subjects. The former was 100-times higher than the latter. PEG precipitation is the most commonly used immunosubtraction method and is widely accepted for the detection of macroprolactin [5] and analytes [6], although it is not perfect. Then, the serum of the patient was precipitated by using PEG. TFT showed FT3 and TSH levels did not change significantly, whereas FT4 levels decreased significantly. These trends were consistent with the clinical manifestations of the patient (Tab. 2).

Patient's THAAb level

The THAAb titre of the patients was more than 100-times that of the control group. The mean value of the T3 antibody (%) in the normal control group was 2.1% and

that in the patient was 4.7%, which was within the normal range. The mean value of the T4 antibody (%) in the normal control group was 0.5% and that in the patients was 59%, which was considerably higher than the normal level.

Discussion

Potential causes of spurious TFT results include nonspecific binding of endogenous circulating factors such as heterophilic antibodies, albumin variants, and THAAbs [7]. THAAb, an analyte autoantibody specific to T4 and T3, is the only antibody reported to interfere with TFT. In theory, only one-step immunoassays are likely to be affected by THAAb interference. While the one-step assay is vulnerable to THAAs, which directly compete with endogenous free T4, a two-step assay employing an intermediate washing step induces a non-competitive reaction that removes the unbound free T4 and interfering factors.

Some studies recommend PEG precipitation to eliminate the interference of THAAbs in serum TFT. As a simple method to remove immunoglobulin, PEG precipitation is important for improving immunoassay accuracy [8].

When TFT results are inconsistent in clinical testing, other methods for investigating immunoassay interference include the following: (a) demonstrating **onlinear [nonlinear?]** response to sample dilution; (c) demonstrating iodothyronine binding to the patient's IgG through electrophoresis or precipitation with anti-IgG; (d) blocking heterophile antibodies with nonimmune serum or blocking tubes; and (e) suppressing patient antibodies with immunosuppressive therapy [9].

In this case, we confirmed immunoassay interference by repeating the analysis on different platforms and demonstrating the presence of THAAbs through PEG precipitation. Antibody interference with serum free thyroxine must be considered when clinical findings and laboratory results show discrepancies. Prompt communication between clinicians and laboratory professionals can avoid unnecessary diagnostic procedures and treatments.

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Conflicts of interest

The authors declare no conflict of interest.

Authors' contribution

All authors designed, analysed, wrote the draft of the manuscript, and approved the final version for publication. All authors agree to accountability for the accuracy and integrity of the work.

Ethical approval

This study was approved by the Ethics Committee of Tianjin Medical University General Hospital. The subject had given written informed consent.

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Table 1. Comparison of the TFT results of different detection platforms

Test item	**SI unit**	**Siemens**	**RR**	**RIA**	**RR**	**Abbott**	**RR**	**Roche**	RR
Total T3	nmol/L	1.01	0.92 – 2.79	Not tested	default	0.98	0.89–2.44	1.35	1.30 – 3.10
Total T4	nmol/L	49.88	58.10– 140.6	Not tested	default	54.34	62.68 – 150.84	61.21	66.00– 181.00

Free T3	pmol/L	0.49	3.50–6.50	0.63	2.07–6.0	0.98	2.63–5.70	0.68	3.10–6.80
Free T4	pmol/L	92.93	11.50–23.50	2.63	6.65–19.9	7.33	9.01–19.05	9.56	12.00–22.00
TSH	mIU/L	> 150	0.30–5.00	> 81	0.7–7.59	> 100	0.35–4.94	> 100	0.27–4.20

RR — reference range; T3 — triiodothyronine; T4 — thyroxine; TSH — thyroid-stimulating hormone

Table 2. Comparison of thyroid function test (TFT) results obtained with the Siemens ADVIA XP testing platform before and after polyethylene glycol (PEG) treatment

	FT3 (pmol/L)	**FT4 (pmol/L)**	**TSH (mIU/L)**
Original serum	0.49	92.93	> 150
PEG-treated serum	0.47	6.84	> 150
Reference interval	3.5–6.5	11.5–23.5	0.3–5.0

FT3 — free triiodothyronine; FT4 — free thyroxine; TSH — thyroid-stimulating hormone