

Positive Interference in Triiodothyronine (T₃) Assay Using a Radioimmunoassay Kit

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Determination of thyrotropin (TSH), total and free thyroxine (T₄) and triiodothyronine (T₃) are widely used for thyroid function evaluation. There have been numerous reports of interference in thyroid hormone immunoassays. Herein, the possible occurrence of interference is investigated for a radioimmunoassay kit of total T₃.

Materials and Methods: A total of 3471 patients were examined through the serum level measurement of TSH, total T₄ and T₃. T₃ analysis was made through a competitive solid-phase radio labeled (¹²⁵I) immunoassay by T₃ Izotop kit (Izotop Co. Budapest, Hungary). The presence of T₃ assay interference was considered probable if the endocrine profile was inconsistent with the clinical picture and/or the obtained value for T₃ showed extreme deviation from normal levels, i.e. above 780 ng/dL. For such patients, the existence of interference was verified by re-measuring T₃ level by another RIA kit (Immunotech kit, Marseille, France).

Results: Among 3471 patients studied, 40 cases (1.2 %) had spuriously high T₃ serum levels with T₃-Izotop kit while normal T₃ levels (132.1±31.0 ng/dL) were observed with T₃-Immunotech kit; the positive interference was more prevalent among women (1.4% vs. 0.5% in men), especially post-menopausal women. Mean serum levels of total T₄ and TSH in the positive interference group were 9.0±2.0 µg/dL and 1.79±1.47 µU/mL, respectively.

Conclusion: In accordance with numerous reports of interferences in thyroid hormone im-

munoassays, the results of our study indicates that both laboratory professionals and clinicians must be vigilant to the possibility of antibody interference in thyroid function assays.

Key Words: Radioimmunoassay, Thyroid function test, Positive interference, Triiodothyronine, Anti-T₃ antibodies

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Introduction

Measurements of thyrotropin (TSH) and of total and free thyroxine (T₄) and triiodothyronine (T₃) are widely used as diagnostic methods for thyroid function evaluation. These measurements are generally based on immunoassay methods. It is well known that immunoassay methods are prone to the "interference" problem,¹ the effect of a substance present in the analytical system, which causes a deviation of the measured value from true value.² In the past 25 years, there have been numerous reports of interferences in thyroid hormone immunoassays.³ The three major possible sources of antibody interference in thyroid hormone immunoassays are autoantibodies,^{4,5} heterophile antibodies,⁶⁻⁸ and rheumatoid factors.^{7,8} Autoantibodies can cause analyte-specific interference in thyroid assays, in contrast to heterophile antibodies

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and rheumatoid factors, which may be responsible for method-specific disturbances in a wide range of immunoassays, including thyroid hormone measurement techniques.³ The importance of interference on clinical laboratory analysis may be evaluated by the frequency with which those lab tests are used in clinical situations and the degree of impact that an incorrect lab result may make on patient care. The high incidence of thyroid diseases, and the serious influence of incorrect laboratory values on clinical decisions, clearly indicates the critical importance of interference diagnosis in thyroid hormone immunoassays. There are a number of ways to recognize the probable presence of assay interference, e.g. lack of agreement between patient's lab results and his (her) clinical condition, markedly differing results given by different analytical methods, the lack of an inverse relationship between serum levels of free thyroid hormones and TSH, extreme deviation from normal or even pathological concentrations, etc.² Applying the above-mentioned criteria, the present study was designed to assess the presence of assay interference for a diagnostic kit of total T₃.

Materials and Methods

The study population included 3471 cases that had been referred by collaborating endocrinologists to the hormone laboratory of the Endocrine Research Center, Shaheed Beheshti University of Medical Sciences for a thyroid function analysis during 2004-2005. Serum levels of TSH and total T₄ and T₃ were measured for each patient. TSH and total T₄ were measured by IRMA and RIA kits (Izotop Co., Budapest, Hungary), respectively. Cases with serum level of TSH below 0.3 µU/mL were excluded from the study. T₃ analysis was made with T₃ Izotop kit. The Izotop T₃-RIA kit had been manufactured by Izotop Co., Budapest, Hungary. The detailed method of laboratory analyses will be described in the next section. The presence of T₃ assay interference was considered prob-

able if the T₃ level was inconsistent with TSH level, according to the endocrinologist, or if the obtained value for T₃ was extremely deviated from normal or even pathologic concentrations, i.e. it was above 780 ng/dL. For such patients, the possibility of T₃ assay interference was investigated by re-measuring T₃ level by Immunotech T₃-RIA kit, manufactured by Immunotech Co. Marseille, France. If the new value of T₃ level obtained by Immunotech T₃-RIA kit was both markedly different from the value obtained by Izotop T₃-RIA kit and consistent with the clinical picture, the case was regarded as T₃ assay interference by the Izotop T₃-RIA kit.

Laboratory analysis. Blood specimens were collected from patients between 8-10 AM after overnight fasting; sera of patients were then prepared by leaving the specimens at room temperature for 10 minutes, followed by centrifugation at 3000 g for 15 minutes. The sera were then either used immediately or stored at -20 °C for less than 7 days. The total concentration of T₃ in each sample was determined using the solid-phase reagent-limited immunoassay with ¹²⁵I-labeled antigen, after its removal from binding proteins through use of 1,8-anilinonaphthalene sulfonate (ANS) and salicylates as displacers and a slightly alkaline pH. This is a heterogeneous competitive immunoassay method in which T₃ from the specimen competes with labeled T₃ for binding to the mouse monoclonal anti-T₃ antibody in coated tubes. After washing, the radioactivity of the bound label was measured by Gamma Counter Wallac Wizard 20101 (Turku, Finland). The coefficients of variation were less than 6.8% and 8.3% for T₃ measurements with Izotop and Immunotech T₃-RIA kits, respectively. For cases with total T₃ above 780 ng/dL as measured by the Izotop kit, more sample dilution necessary for accurately determination of total T₃ level was not performed. Statistical data were presented as mean and standard deviation and the mean values were compared between groups using t test. Since for

the interference group with total T₃ above 780 ng/dl, the accurate values of total T₃ were not available, the statistical comparison of total T₃ levels (as measured by the Izotop kit) between the interference and total groups was made according to the non-parametric Mann-Whitney U test; level of significance was set at 0.05.

Results

A total of 3471 patients, with the mean±SD age of 37.4±16.3 years participated in the study; female to male ratio was 3:1 in the studied population. The mean age of men, 40.4±18.3 years, was significantly higher than that of women, 36.5±15.6. Table 1

demonstrates some characteristics of the studied population.

Of 3471 patients, according to the Izotop T₃-RIA kit, 40 cases (36 women, 4 men), had high T₃ serum levels inconsistent with their clinical pictures and/or extremely deviated from normal values. The serum level of T₃ was spuriously high (above 780 ng/dL) in 37 cases and 696, 614 and 372 ng/dL in three remaining cases. In all these 40 cases, T₃ level was in the normal range according to T₃-Immunotech kit (with a mean±SD of 132±31 ng/dL), in accord with their clinical pictures. The difference between Izotop and Immunotech results was statistically significant in this group.

Table 1. Characteristics of the population studied, and the subgroup with positive interference in T₃ determination by T₃-RIA Izotop kit

	Total Sample	Group with interference
Number of subjects	3471	40
Age (years)	37.4±16.3	38.8±15.0
Female to male ratio	3±1	9±1
Total T ₄ (µg/dL)	8.9±2.8	9.0±2.0
TSH (µU/mL)	2.19±1.79	1.79±1.47
Total T ₃ by Izotop kit (ng/dL)	127±43	>780*†
Total T ₃ by Immunotech kit (ng/dL)	-	132±31

* P<0.001; †. For cases with total T₃ above 780 ng/dL by the Izotop kit, more sample dilution necessary for the accurate measurement of T₃ level was not performed. Therefore, the statistical comparison between the interference and total groups was made according to the non-parametric Mann-Whitney U test

The frequency of positive interference occurrence was 1.2% in total group. The prevalence of positive interference was significantly higher in women (1.4% in women vs. 0.5% in men). Mean age of patients with positive interference in T₃ determination by Izotop T₃-RIA kit was 38.8±15.0 years, not significantly different from the corresponding value of 37.4±16.3 for the total sample. However, the age structure of two groups was different. Specifically, while 72% of women in the total sample were aged under 45 years, 9% of them were between 45 and 50 years old and 19% older than 50 years; the

corresponding values for women in the positive interference group were 55%, 15% and 30%, respectively. Hence the frequency of positive interference occurrence was 2.4% in women aged over 50 years (post-menopausal ages), 2.1% in 45-50 years old women (around menopause) and 1.1% in women aged below 45 years.

The mean serum level of TSH was 1.79±1.47 µU/mL in total group, not significantly different with the interference group. The mean total serum value of T₄ was 9.0±2.0 µg/dL in the total sample, again not significantly different to the interference

group. This remained true even when the comparisons of TSH and total T₄ were restricted to female cases of the two groups.

Discussion

The above-mentioned evidence clearly indicates the presence of positive interference(s) in some T₃ assays made with Izotop T₃-RIA kits. The frequency of positive interference in serum total T₃ measurement by this kit was found to be 1.2%. The positive interference was more prevalent among women (1.4% vs. 0.5% in men), especially post-menopausal women.

The major possible sources of such positive interferences in T₃ immunoassays are autoantibodies against thyroid hormones, heterophile antibodies, rheumatoid factors, and T₄ cross reactivity. Autoantibodies to thyroid hormones were first described by Robbins et al. in 1956.⁹ The prevalence of thyroid hormone autoantibodies (anti-T₃ and anti-T₄ antibodies) among the overall population is between 0 and 1.8 percent,¹⁰ but higher (up to ~10%) in hypothyroid, hyperthyroid, and non-thyroid autoimmune patients.¹¹ The direction of bias caused by the presence of thyroid hormone autoantibodies depends on many factors such as the single- or double-antibody procedure, one- or two-step assay, molecular features of the tracer used, as well as the immunological features, like autoantibody titer, specificity, and affinity.³ In the solid-phase reagent-limited single-antibody technique that we have used in the present study, the presence of autoantibodies may cause falsely high hormone concentrations because the tracer is bound by the autoantibodies as well as by the capture antibodies. Hence, after the separation process, an abnormally low amount of tracer is detected, and the apparent concentration of hormone will be spuriously high. The positive interference observed in about 1.2% of patients in the present study may therefore have been caused by these relatively common thyroid hormone autoantibodies. If so, the

lack of interference in T₃ determination by the second kit (Immunotech T₃ RIA kit) may be assigned to much higher affinity of Immunotech primary antibody for tracer so that, in the case of T₃ determination by Immunotech T₃ RIA kit, thyroid hormone autoantibodies existing in the sample will contribute little in tracer binding and the positive bias caused by autoantibodies will be minimal.

Heterophile antibodies have been reported to be present in 30-40% of patient samples.¹² Heterophile antibodies are known to interfere in a wide spectrum of immunoassays, including thyroid hormone measurements.^{7,8} These antibodies interfere with both reagent-limited and reagent-excess assays.² In the reagent-limited assays like that used in the current study, the presence of heterophilic antibodies lowers the number of available binding sites on the primary antibody, e.g. through steric hindrance and preventing the binding of antigen, hence leading to a positive bias. Rheumatoid factors also may behave like heterophilic antibodies and exhibit nonspecific binding to the analytical antibodies.^{7,8} These IgM-isotype antibodies against the Fc portion of human IgG are present in low concentrations in about 5% of the normal population.² They interfere with reagent-limited assays, by binding to the Fc region of the immunoglobulin and probably blocking the analyte-specific binding sites. So, the presence of heterophilic antibodies and/or rheumatoid factors should be considered as possible explanations of the observed positive interference in the current study, although this mechanism seems to be much less likely than the presence of thyroid auto-antibodies. The lack of interference in T₃ determination by Immunotech T₃-RIA kit would then be explained by the differences in primary antibodies of these two kits, hence the different affinities of heterophilic antibodies and/or rheumatoid factors for those antibodies.

In the current study, the cases were first evaluated by the use of the Izotop kit and if any doubt of positive interference arose, re-measurement of T₃ was done by the second

kit (Immunotech kit). Therefore, we should limit our claim to the positive interference revealed in some cases by the Izotop kit. This does not however exclude the possible occurrence of interference by the Immunotech kit in some of the remaining cases

In conclusion, the occasional presence of positive interference in T₃ immunoassay with Izotop T₃-RIA kit was found in the present study. Probable explanations of such interferences were presented, but the exact cause of interference has yet to be determined. In ac-

cordance with numerous reports of interferences in thyroid hormone immunoassays in the past 25 years, the results of our study indicate that both laboratory professionals and clinicians must be vigilant to the possibility of antibody interference in thyroid function assays.

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