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A Pseudo-Hyper Triiodothyroninemia Caused by Heterophile Antibodies Interference in Radio Immunoassay

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Introduction: Since mouse antibodies are commonly used in diagnostic kits, their presence including auto antibodies/heterophile antibodies in some patients' sera has been known as a potential source of interference and false immunoassay results for a long time.
Case Presentation: This is a case report of a 49 year-old woman presenting with nonspecific symptoms suspected to hyperthyroidism. Her total triiodothyronine (T3) test was more than twice of the expected value, exclusively by radio immunoassay (RIA) method. Interferences due to the protein binding disorders was ruled out using radio-labeled T3, binding to the separated binding proteins by electrophoresis. Presence of auto antibody ruled out by a manual (in house) method. In this manual method, anti human IgG and IgM coated wells used for auto antibody stripping. In addition animal serum used as blocking reagent. Precipitating technique by polyethylene glycol (PEG) showed a false-positive result in T3 assay caused by a heterophile IgG class antibody.
Discussion: In our review of literatures, so far, this is the first case report of false-positive interference, exclusively in total T3 assay due to Heterophile antibody.

Keywords: False Positive Reactions; Microscopy, Interference; Hyperthyroxinemia, Familial Dysalbuminemic; Antibodies, Heterophile

1. Introduction

Many endogenous antibodies exhibit a potential interference with immunoassays because mouse antibodies are commonly used in diagnostic kits. Major types of antibodies that interfere with immunoassays are called heterophilic antibodies. They consist of natural and auto antibodies. Both types are usually weak which are produced against poorly-defined antigens and interfere with immunoassays. Human anti-animal antibodies, developed as a result of treatments with animal immunoglobulins, are produced against well-defined antigens (1). Despite the manufacturers' attempts to compensate this source of error, it is still present. Hence, laboratory technicians occasionally encounter interferences causing false positive or false negative results. If such false results remain unrecognized, they may result in incorrect diagnoses and consequently inappropriate therapeutic interventions, which may not only be costly but can also endanger the patients' health. Therefore, an explanation of the immunoanalytical basis of these assays could be effective in raising the awareness regarding such cases (2, 3).

2. Case description

This is the case report of a patient (49 year-old woman), presenting with non-specific symptoms and signs (palpitation, anxiety, mild weight loss and grade 1 goiter) suspected to hyperthyroidism, several years ago. Following the thyroid function tests, a significant increase of the total T3 level, twice of the normal value, was observed. The patient was followed up because of the high level of total T3 and underwent certain prevocational (thyrotropin releasing hormone or TRH test) and suppressive (T3 suppression) tests. Although thyroid scan and radioactive iodine uptake test were suggestive of a euthyroid state, they did not lead to determination of the cause of the total T3 level elevation. Following the results of primary evaluations, indicating that it might be a hereditary autosomal dominant disorder (familial dysalbuminemic hypertriiodothyroninemia or FDH-T3) (4), the patient and her first-degree relatives underwent investigations. Obtained data including clinical history, physical examination and laboratory investigations were collected. Hormonal assays were performed by Radio Immunoas-

Implication for health policy/practice/research/medical education:
This case report is a warning for clinicians and para clinicians to notice the HAMA interference in laboratory test results such as thyroid panel tests. Being aware of this interference prevents false results and incorrect treatments, patients' health will be maintained.

say (IMMUNOTECH RIA T3, France and Kavoshyar total T3 RIA, Tehran, IR Iran) and ELISA (DRG T3 EIA, USA) methods, separately. According to the different obtained results in T3 assays of these two immunoassay methods, recovery and parallelism accuracy tests were performed. Complimentary tests were performed including: serum protein binding proteins assessment (both qualitative and quantitative), routine thyroid auto-antibodies (Anti-thyroid peroxidase and anti-thyroglobulin) using ELISA method (TPO Orkit LABODIA, Switzerland), presence or absence of Anti-T3 auto antibodies by the in-house method, and removal (by wells coated with antihuman IgG and IgM) and blocking (by different animal serums that usually neutralize anti-animal antibodies) techniques for elimination of the heterophile antibodies.

3. Dicussion

Thyroid function tests were performed using two RIA and ELISA methods, for the patient and her first-degree relatives, the results of which were normal in all the relatives. As mentioned, high total T3 level (with both RIA kits: 385 ng/dL by IMMUNOTECH RIA T3, France; 430 ng/dL by Kavoshyar T3 RIA, Tehran, IR Iran; but free T3 was 11.9 pmol/L by BYK-Sangtec RIA coat FT3-Germany) was only reported in the patient sample, so complementary tests focused on the patient serum. Total T3 was abnormally high only in RIA but not in the ELISA method. Accuracy (recovery and parallelism) tests also showed that here, the results of RIA may not be correct. Serum albumin concentration was normal. Serum iodoprotein evaluation with SDS-PAGE method and silver staining procedure, which is a sensitive method used for this purpose in protein concentrations of ng/mL, did not show any abnormality in the binding proteins compared to the normal sera. Separate band radioactivity after acetate cellulose electrophoresis, following incubation of the patient serum with radio-labeled T3, did not indicate any abnormality compared to the control samples. Radioactivity of the precipitant after addition of polyethylene glycol (PEG) to the serum which had been incubated with radio-labeled T3, did not show any significant difference compared to the controls, both of which were less than 5%. After the addition of PEG to the patient serum, the sample was centrifuged and the supernatant was examined for the T3 level. Following this procedure T3 was normalized, so probably the experiment eliminated the interfering factor (Table 1).

Incubation of the patient serum with animal serum results in T3 normalization, only using mouse serum in a non-reproducible manner. Even using the outbreed mice pool could not decrease the interference. After incubation of the patient serum with rabbit antihuman IgG-coated wells, T3 assay was corrected and this was reproducible, while goat antihuman IgM-coated wells could not decrease the T3 level significantly (Table 2).

The presence of antibodies in some patients' serum is a potential source of interference in immunoassays such as Radio Immunoassay (RIA). Antibodies that interfere with immunoassays are called heterophile or heterophilic antibodies that are usually developed after exposure to animal's serum protein, although sometimes there is no determinable cause for their development (5, 6). Heterophile antibodies interference is a common problem in many immunoassay methods and not limited to the hormonal assay. Lippi et al. in a systematic review described it in details (7). Our data reveled that probably the interfering antibody in our case belonged to the IgG class and was an anti-idiotype antibody. In RIA methods, the radio labeled analyte is traced with specific and high affinity antibodies. In thyroid function tests, if auto antibodies (anti-T4 and anti-T3) are present, they may be the source of interference. However, heterophilic antibodies often interfere in the two-site methods such as IRMA (8-12). Theoretically, heterophilic antibodies can interfere in RIA only if they are anti-idiotype (high affinity) or present in high concentrations. Considering the tests carried out and ruling out the latter possibility, the interfering agents in our patient probably were anti-idiotype antibodies. This is the first case report of interference in T3 assay exclusively, due to a heterophile antibody. Before this, only Fiad et al. (10) reported interference in all thyroid function tests except T3RU caused by heterophile antibodies in 1994, but until present no other similar case has been report. Interference of auto antibodies in the T4 and T3 assays as well as heterophile antibodies in the TSH assay (two-site methods) has been reported repeatedly, especially in hyperthyroidism (8-13).

Table 1. T3 Level of the Patient and Control Sera Before and After the PEG Precipitation

	T3 Level, ng/dL	
	Patient	**Control**
Before PEG treatment	385	172
After PEG treatment	180	165

Table 2. The T3 Level in the Patient Serum Before and After the Incubation With Anti-Human IgG and IgM

	Before Incubation	**After Incubation**	
		With Rabbit Antihuman IgG	**With Goat Antihuman IgM**
Serum patient T3 Level, ng/dL	385	150	305

As a conclusion, clinicians must be aware of the interference in immunoassay methods and the occasion this is suspected (when the results of thyroid function tests appear to be internally inconsistent or incompatible with the clinical presentation). The most important strategy is the routine communication between laboratory professionals and clinicians (8, 9). We reported a false positive interference in triiodothyrotropin (T3) using radioimmunoassay kit with 1.15% rate or 40 false positive results among 3471 samples (14). In this way, any discrepancy between clinical and laboratory findings can be followed up. When there is interference probability as a possible cause, the laboratory should repeat the suspected test to confirm the findings. If the finding is still persistent, then: (a) both the clinical findings (the disease state and treatment) and specimen related information (sample and storage conditions, and results of any other assays, especially immunoassays done on the same specimen) must be documented, and (b) reevaluation must be done using another comparable method. In addition, for T3, T4 and TSH, nonlinearity of sample dilutions may indicate the interference. This is not recommended for free hormone assays; however, parallelism test or serial dilutions can reveal the assay accuracy (15, 16). Our case report and similar data highlight the persistent and intrinsic nature of this problem, despite the manufacturers attempts to compensate this source of error; thus, clinical laboratories should be alert to the assay interference by antibodies, irrespective of their natures.

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Authors' Contribution

Majid Valizadeh prepared the manuscript. Fereidun Azizi designed the project. Mehdi Hedayati performed the laboratory assessment and was responsible for correspondence of the manuscript.

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References

1. Bolstad N, Warren DJ, Nustad K. Heterophilic antibody interference in immunometric assays. *Best Pract Res Clin Endocrinol Metab*. 2013;**27**(5):647–61.
2. Sanmartin N, Garcia C, Bugier S, Malfuson JV, Chianea D, Renard C, et al. [Heterophilic antibodies: be carefull!]. *Ann Biol Clin (Paris)*. 2013;**71**(4):475–80.
3. Diamandis EP. Immunoassay interference: a relatively rare but still important problem. *Clin Biochem*. 2004;**37**(5):331–2.
4. Sunthornthepvarakul T, Likitmaskul S, Ngowngarmratana S, Angsusingha K, Kitvitayasak S, Scherberg NH, et al. Familial dysalbuminemic hypertriiodothyroninemia: a new, dominantly inherited albumin defect. *J Clin Endocrinol Metab*. 1998;**83**(5):1448–54.
5. Frost SJ. More on heterophile and human anti-animal antibodies. *Clin Chem*. 1999;**45**(11):2042–3.
6. Kaplan IV, Levinson SS. When is a heterophile antibody not a heterophile antibody? When it is an antibody against a specific immunogen. *Clin Chem*. 1999;**45**(5):616–8.
7. Lippi G, Aloe R, Meschi T, Borghi L, Cervellin G. Interference from heterophilic antibodies in troponin testing. Case report and systematic review of the literature. *Clin Chim Acta*. 2013;**426**:79–84.
8. Kricka LJ. Human anti-animal antibody interferences in immunological assays. *Clin Chem*. 1999;**45**(7):942–56.
9. Despres N, Grant AM. Antibody interference in thyroid assays: a potential for clinical misinformation. *Clin Chem*. 1998;**44**(3):440–54.
10. Brennan MD, Klee GG, Preissner CM, Hay ID. Heterophilic serum antibodies: a cause for falsely elevated serum thyrotropin levels. *Mayo Clin Proc*. 1987;**62**(10):894–8.
11. Schaison G, Thomopoulos P, Moulias R, Feinstein MC. False hyperthyrotropinemia induced by heterophilic antibodies against rabbit serum. *J Clin Endocrinol Metab*. 1981;**53**(1):200–2.
12. Bjerner J, Nustad K, Norum LF, Olsen KH, Bormer OP. Immunometric assay interference: incidence and prevention. *Clin Chem*. 2002;**48**(4):613–21.
13. Jahagirdar VR, Strouhal P, Holder G, Gama R, Singh BM. Thyrotoxicosis factitia masquerading as recurrent Graves' disease: endogenous antibody immunoassay interference, a pitfall for the unwary. *Ann Clin Biochem*. 2008;**45**(Pt 3):325–7.
14. Rezaei-Ghaleh N, Hedayati M, Ordookhani A, Azizi F. Positive Interference in Triiodothyronine (T3) Assay Using a Radioimmunoassay Kit. *Int J Endocrinol Metab*. 2007:142–146.
15. Fiad TM, Duffy J, McKenna TJ. Multiple spuriously abnormal thyroid function indices due to heterophilic antibodies. *Clin Endocrinol (Oxf)*. 1994;**41**(3):391–5.
16. Hedayati M, Yazdanparast R, Azizi F. Determination of human tumor necrosis factor alpha by a highly sensitive enzyme immunoassay. *Biochem Biophys Res Commun*. 2001;**289**(1):295–8.