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Case Report

An Adolescent with Prolactinoma or Factitious Hyperprolactinemia: Role of Laboratory Immunoassay

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Abstract

Introduction: Immunoassays, particularly when immunometric, may show falsely low/elevated levels due to interference from non-specific antibodies in the serum [1].

Case Description: A 13 year old male evaluated for short stature was found to have elevated serum prolactin (PRL) 101.8 ng/mL (5 - 18 ng/mL) and FSH levels 61.1 mIU/mL (1.6 - 9.7 mIU/mL). Brain MRI showed a 3 mm microadenoma. The PRL level remained high despite being on Cabergoline, with good compliance, for 3 months. Factitious hyperprolactinemia due to interfering antibodies (HA) was suspected.

The serum sample was re-analyzed using 1. Serial dilution with the same analyzers 2. Heterophile blocking tube (HBT, Scantibodies) and non-specific antibody blocking tube (NABT, Scantibodies) 3. Different platforms in reference lab ARUP (prolactin on the ADVIA Centaur and FSH on the Roche COBAS). The results confirmed our suspicion.

Conclusion: It is important to look for interfering antibodies when the clinical picture does not correspond to the lab findings, to avoid unnecessary investigations and treatments.

Keywords: *Heterophile Antibodies; Non-Specific Antibodies; Interfering Antibodies; Factitious Hyperprolactinemia*

Abbreviations

PRL: Prolactin; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone; RF: Rheumatoid Factor; HAMA: Human Anti-Mouse Antibodies; NABT: Non-Specific Antibody Blocking Tube; HBT: Heterophile Blocking Tube; TSH: Thyroid Stimulating Hormone; PSA: Prostate-Specific Antigen; SHBG: Sex Hormone-Binding Globulin; HCG: Human Chorionic Gonadotropin; PTH: Parathyroid Hormone; AMH: Anti-Mullerian Hormone

Introduction

In 1960 Yalow and Berson developed RIA, using radioactive tracers and antibodies to measure insulin. Over the years the technique of immunoassays has evolved and now variations of RIA like ELISA, chemiluminescence and others, which use enzymes or fluorescent markers, are widely used in clinical laboratories.

An immunoassay measures the concentration of an analyte in a solution using an antibody. Immunometric immunoassays which rely on antigen-antibody reactions are subject to interference with various endogenous antibodies (Figure 1). Interfering antibodies are present in up to 40% of the general population [2]. Exposure to mice and its products, vaccines, blood transfusions, autoimmune diseases, dialysis and maternal transfer have been proposed as the sources of these antibodies [2,3]. These interfering antibodies cause no apparent clinical problems; however, they can yield falsely elevated or diminished results on immunoassays [4].

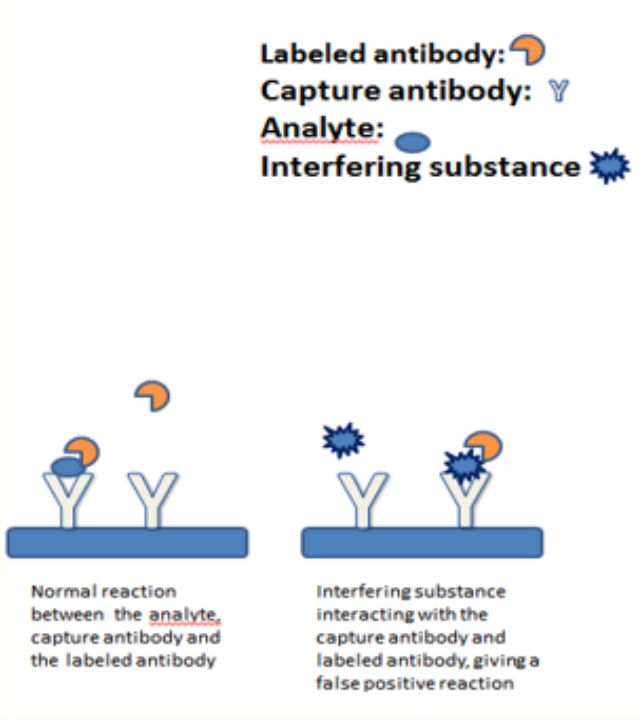

Figure 1: *Mechanism of interference of the interfering antibodies in Immunometric assays.*

We report a case of an adolescent male initially diagnosed with prolactinoma, who was found to have falsely elevated prolactin levels due to interfering antibodies.

Case Report

A 13 year old male was referred to our endocrine clinic for short stature. He had always been short and healthy. His mid parental height was 171.6 cm (67.5 inches). His height at presentation was 142.3 cm (56 inches, 5th%) and weight 34.5 kg (7th%). Physical exam was prepubertal. His immunization records were up to date. There was no history of exposure to blood transfusions, immunoglobulins, rats and no infectious mononucleosis.

Biochemical data: Normal hemogram, metabolic profile, thyroid function tests and growth factors. The serum prolactin was elevated 101.8 ng/ml (4.5 - 23 ng/ml). His FSH (61.1 mIU/ml) was elevated, LH was normal (1.0 mIU/ml). Androgens were normal for pubertal status (Testosterone: 8 ng/dl, DHEAS: 112 mcg/dl). Karyotype was 46 XY.

Radiological data: Bone age was 11 years for a chronological age of 13 years and 3 months with a predicted adult height of 68 inches. MRI showed a 3 mm pituitary microadenoma. Based on the serum prolactin level and the MRI findings, he was diagnosed with microprolactinoma.

Treatment: He was initially started on oral Cabergoline 0.25 mg twice a week, which was gradually increased to 1 mg twice a week over 3 months. However, serum PRL levels remained unchanged despite good compliance with the medication. A clinical suspicion of factitious hyperprolactinemia was raised.

Interference due to possible heterophile/non-specific antibodies was suspected and the biochemical findings were reviewed in detail. The initial laboratory analysis was performed in-house on the Ortho Vitros 5600 which uses a two-site immunometric immunoassay with a sheep monoclonal capture antibody and a mouse monoclonal detection antibody for both prolactin and FSH. Due to the suspicion of falsely elevated prolactin in this patient, further studies were performed to determine whether there was interference in the immunoassay technique used to measure prolactin. As FSH was also inexplicably elevated, similar investigations were performed for FSH.

Additional Biochemical Analysis

1. **Serial dilutions**: These were performed to observe whether dilution was linear. Dilutions of 1:10 and 1:100 were non-linear for both prolactin and FSH suggesting interference in the assay (Table 1).
2. **Blocking agents**: The patient's serum was pretreated with blocking reagents to see whether this had any effect on the values for prolactin and FSH. Two blocking reagents used were HBT [Heterophile Blocking Tube (Scantibodies Laboratory, Inc, Santee, CA)] and NABT [Non-Specific Antibody Blocking Tube (Scantibodies Laboratory Inc, Santee, CA)]. Both the HBT and the NABT significantly decreased prolactin and FSH. Prolactin decreased from 105.6 ng/ml to 4.7 ng/ml with HBT and < 1.4 ng/ml with the NABT. FSH decreased from 61 mIU/ml to 20.6 mIU/ml with HBT and 4.6 mIU/ml with NABT (Table 1). While these blocking assays do not provide reliable quantitation of prolactin and FSH, they do demonstrate the likelihood of antibody interference in this patient.
3. **Use of different platform**: The patient's serum was then forwarded to a reference laboratory (ARUP Laboratories, Salt Lake City, Utah) for testing on a different platform. ARUP uses the Siemens Advia Centaur assay for prolactin and the Roche Cobas assay for FSH. The Advia prolactin assay is a two-site immunoassay with a mouse monoclonal detection antibody and a goat polyclonal capture antibody. The Roche FSH assay is a two-site immunoassay which uses a mouse monoclonal capture antibody and a mouse monoclonal detection antibody. Results of prolactin testing at ARUP were 0.3 ng/ml (Reference Range 2.1 - 17.7 ng/ml) compared 105.6 ng/ml in our laboratory. The results for FSH at ARUP were 3.4 mIU/ml (Reference Range 1.7 - 7.4 mIU/ml) compared to 61.3 mIU/ml in our laboratory (Table 1).

	Prolactin (5 - 18 ng/ml)	FSH (1.6 - 9.7 mIU/ml)
	In-house	**In-house**
0 months	109.2	61.1
1 month	97.5	54.5
3 months	105.6	61
4 months (1 month after discontinuation of Cabergoline)	105.9	63
Dilution studies- at 3 months		
1:2	134.4	105.4
1:10	179.2	54.2
1:100	260.9	591
Immunoassay with Blocking tubes - at 3 months		
HBT	4.7	20.6
NABT	< 1.4	4.6
Immunoassay using different platform - at 3 months		
In-house	105.6	61.3
Reference lab ARUP	0.3	3.4

Table 1: *Table showing the patient's serum Prolactin and FSH values using different lab assays. HBT: Heterophile Blocking Tube; NABT: Non-Specific Antibody Blocking Tube*

It was difficult to determine whether there is any species specificity of the interfering antibody as these assays all use at least one anti-mouse antibody.

Notably, this patient's Rheumatoid Factor (RF) was also elevated at 29 IU/ml (Reference Range < 12 IU/ml). Rheumatoid Factor is an antibody that binds to the Fc portion of IgG. RF has been known to cause falsely elevated D-dimer results in some immunoassays and may be contributing to these falsely elevated results for prolactin and FSH in our patient. In fact, newer assays use the F(ab)2 portion of that antibody for detection of d-dimer in order to avoid interference from rheumatoid factor.

Discussion

There have been multiple case reports in adults about endogenous antibodies interfering with different immunoassays (TSH, Troponin I, PSA, LH, FSH, SHBG, HCG, prolactin, testosterone, PTH, AMH) [2,5-12]. We are aware of only one case report in pediatrics where a 10 y/o male was found to have high TSH levels with normal FT4 levels, TSH not responding to levothyroxine therapy for 2 years, after which heterophile antibodies were found to be the reason for the elevated TSH [2].

Recently, another case has been reported in a neonate [unpublished data] who was diagnosed with congenital hypothyroidism on new born screening (TSH > 555, normal T4), and was started on levothyroxine treatment. The baby had a normal gland on ultrasound with normal uptake. In view of this discrepancy between the imaging and the lab findings, maternally transferred antibodies were suspected. The mother's TSH was found to be 178 IU/L, but she had no history of thyroid disease. Both the mother and baby's samples showed non-linear dilution and the levels did not normalize on using HBT/NABT but normalized on using a different platform (Siemens Centaur chemiluminescence assay), confirming maternal transfer of interfering antibodies.

Although several reports of single hormone and less commonly multiple hormone assay interferences have been found in adults, only a handful of cases have been reported in children. The reason for this extreme rarity in children remains to be elucidated. It may be possible that the interfering antibodies develop over time or that the testing for these hormones is infrequent in children as compared to adults [8]. Ours is a rare case of a pediatric patient with non-specific antibodies interfering with multiple assays (prolactin, FSH and RF), giving a falsely high level for all of them.

The substances that interfere with immunoassays include heterophile antibodies, human anti-animal antibodies, autoanalyte antibodies, rheumatoid factors and other non-specific antibodies which have structural similarities and can cross-react with the antibody [3]. Heterophile antibodies are low avidity antibodies, which occur naturally and do not require exposure to any immunogen; human anti-animal antibodies (HAAA) are high avidity antibodies produced after exposure to a specific immunogen (anti-mouse being the most common). These can lead to either falsely elevated or falsely low analyte concentrations depending on the site of interference.

When interfering antibodies are suspected, dilution studies can be performed to determine if the sample dilutes in a non-linear fashion. This is a common observation in immunoassay interference due to heterophile antibodies or Human Anti-Animal Antibodies (HAAA) and may be due to the heterogeneity of the interfering antibodies and steric differences as interfering antibodies are diluted.

The patient's serum can be pre-treated with blocking reagents. Two blocking reagents can be used: HBT and NABT. The HBT contains specific binders which inactivate heterophilic antibodies. The NABT contains immunoglobulins that bind to non-specific antibodies in the sample and prevent them from interfering in antibody detection immunoassays.

Additionally, the sample can be tested using different platforms [3,4,8]. Other approaches for detecting whether unexpected results are due to interfering antibodies include polyethylene glycol (PEG) precipitation. PEG will precipitate antibodies present in the patient's serum or plasma. The remaining specimen can then be retested for the analyte of interest. There are also tests available to detect the presence of HAMA (Human Anti-Mouse Antibodies) which can be informative if they are positive, but may not be informative when negative. Lastly, other methods such as mass spectrometry can be used for analysis. Analysis by mass spectrometry avoids the reliance on an antigen-antibody interaction to detect an analyte.

This is important to identify on time as false levels often lead to even more investigations and unnecessary treatments, increasing the burden on healthcare and causing anxiety for the patient and the family. In our patient the high prolactin level led to a MRI of the brain which revealed a microadenoma and initiation of treatment with cabergoline for almost 3 months.

Conclusion

This is a rare case demonstrating falsely elevated serum PRL and FSH levels due to analytic interference by heterophile/ non-specific antibodies using immunoassays leading to the misdiagnosis of a prolactinoma. It is important to recognize the possibility of interfering antibodies when the lab findings do not fit the clinical picture in order to prevent unnecessary investigations and treatments which increase the burden on healthcare and cause anxiety for the patient and the family. In addition, knowledge of laboratory assays and its interpretation must be obtained from experts in the field in such cases.

Conflict of Interest

None of the authors have any conflict of interest.

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