JCEM Case Reports, 2023, **1**, 1–3
https://doi.org/10.1210/jcemcr/luad029
Advance access publication 23 March 2023
Case Report

Insulin Immunoassay Interference Due to Human Antimouse Antibodies in a Patient With Ketotic Hypoglycemia

Meghan Craven,[1] Katherine Lord,[2,3] Karla F. Leavens,[2,3] and Diva D. De Leon[2,3]

[1]Division of Diabetes and Endocrinology, Department of Pediatrics, Texas Children's Hospital, Baylor College of Medicine, Houston, TX 77030, USA
[2]Division of Endocrinology, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA
[3]Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA

Correspondence: Diva D. De Leon, MD, MSCE, Division of Endocrinology and Diabetes, Children's Hospital of Philadelphia, Buerger Center for Advanced Pediatric Care, 3500 Civic Center Blvd, Rm 12128, Philadelphia, PA 19104, USA. Email: deleon@chop.edu.

Abstract

Misinterpretation of common endocrine hormonal immunoassays can distort the clinical picture and lead to unnecessary medical workups. Potential assay inference is important to recognize when the clinical presentation and laboratory evaluation are inconsistent. This is demonstrated by the case of an 18-month-old girl who initially presented with ketotic hypoglycemia and was found on diagnostic fasting evaluation to have the triad of hypoglycemia, inappropriately high insulin levels, and low C-peptide levels—point-of-care glucose 43 mg/dL (2.39 mmol/L) (confirmatory 52 mg/dL [2.89 mmol/L]), insulin 48.1 μIU/mL (334 pmol/L), and C-peptide 0.2 ng/mL (0.07 nmol/L) concerning for factitious insulin (insulin:C-peptide ratio 4.77). On repeat diagnostic fast, insulin assays measured by liquid chromatography–mass spectrometry were incongruent with prior testing by immunoassay, demonstrating a falsely elevated insulin level when measured by immunoassay, likely due to human antimouse antibody interference (HAMA 181 ng/mL). This case represents a diagnostic challenge in which is it imperative to recognize possible immunoassay interference. It is critical to establish the difference between insulin assay interference and factitious insulin through use of alternative laboratory methods as misdiagnosis could lead to the serious implication of Munchausen by proxy resulting in the removal of a child from their home and potentially parents being charged with a crime.

Key Words: insulin assay, human antimouse antibodies, hypoglycemia, hyperinsulinism

Abbreviations: BOHB, β-hydroxybutyrate; ED, emergency department; EMS, emergency medical services; HAMA, human antimouse antibody; IGF-1, insulin-like growth factor-1; LC, liquid chromatography; MS, mass spectrometry; POC, point-of-care.

Introduction

Various laboratory methods are used to assess endocrine problems during patient evaluations, including when patients present with hypoglycemia. Hormonal immunoassays are most commonly used as well as more recently mass spectrometry (MS). However, despite advancements in these laboratory techniques, pitfalls in the accuracy of endocrine testing remain and can sometimes distort the clinical picture [1]. As previously reported in the literature, falsely elevated hormone levels due to assay interference caused by heterophile antibodies have led to unnecessary workup including discordant measurements in thyrotropin, adrenocorticotropin, follicle-stimulating hormone, parathyroid hormone, insulin-like growth factor-1 (IGF-1), prolactin, β-human chorionic gonadotropin, and calcitonin levels [2, 3].

In regard to the workup for a toddler presenting with hypoglycemia, artificial hypoglycemia can occur, often resulting from either improper collection of blood samples or interfering substances in the blood, such as medications or a high hematocrit, but to our knowledge, insulin assay interference has not been previously reported. This inference would be important to recognize, as the differential for an inappropriately elevated insulin level includes factitious hypoglycemia resulting from deliberate insulin injection (Munchausen by proxy), that may escape proper diagnosis for some time. Factitious hypoglycemia is typically best diagnosed by the triad of hypoglycemia, inappropriately high insulin levels, and low C-peptide levels [4, 5]. In cases of factitious hypoglycemia due to exogenous insulin administration, as with any other insulin excess disorder, ketones are expected to be suppressed. We present a case of the triad of hypoglycemia, inappropriately high insulin levels, and low C-peptide levels with an inexplicably elevated β-hydroxybutyrate (BOHB) level.

Case Presentation

On her initial presentation, an 18-month-old girl developed altered mental status for which her father called emergency medical services (EMS). On arrival to the emergency department (ED), her point-of-care (POC) glucose was 38 mg/dL (2.11 mmol/L). At that time, she had been fasting for approximately 16 hours and urinalysis showed large (> 80 mg/dL) ketones. Her weight was 10.5 kg (41st percentile; $Z = -0.23$),

Received: 16 December 2022. **Editorial Decision:** 28 February 2023. **Corrected and Typeset:** 23 March 2023

length 82 cm (50th percentile; $Z = 0$), and body mass index 15.62 kg/m^2 (50th percentile; $Z = -0.15$), all appropriate for her age. She was admitted for monitoring without further hypoglycemia and discharged home with a presumed diagnosis of idiopathic ketotic hypoglycemia.

The patient then re-presented 11 days later with a similar episode of altered mental status in which she appeared tired, uninterested in eating, and difficult to arouse. There was no known prolonged fasting at the time. Her father appropriately treated her with apple juice and called EMS. On EMS arrival her POC glucose was 120 mg/dL (6.66 mmol/L), which dropped to 88 mg/dL (4.88 mmol/L) in approximately 20 minutes by the time of arrival to the ED. She was once again admitted and underwent a diagnostic fast. She was discharged home stable with results pending.

Diagnostic Assessment

The diagnostic fast showed the following critical sample at approximately 12 hours of fasting: POC glucose 43 mg/dL (2.39 mmol/L) (confirmatory 52 mg/dL [2.89 mmol/L]), HCO_3 18 mmol/L, lactate 2 mmol/L, BOHB 3.59 mmol/L, cortisol 10.5 µg/dL (290 nmol/L), growth hormone 6.66 ng/mL, IGF-1 94 ng/mL, ammonia 33 µmol/L, insulin 48.1 µIU/mL (334 pmol/L)and C-peptide 0.2 ng/mL (0.07 nmol/L) (Table 1 shows the expected values for a critical sample during hypoglycemia in a normal child). Given an insulin–to–C-peptide ratio of greater than 1 (4.77), concern for exogenous insulin was raised. When the family arrived home, they were called to return to the ED based on the results of the diagnostic fast. The patient's maternal grandfather was being treated for diabetes but was not on insulin at the time. There were no other known exposures.

When the child re-presented to the emergency department, she was asymptomatic with euglycemia. On admission and repeat fasting of approximately 24 hours, her glucose was 41 mg/dL (2.28 mmol/L), with an insulin level of 42 µIU/mL (292 pmol/L) and C-peptide of 0.4 ng/mL (0.13 nmol/L) (ratio of 2.2). Child protective services was called at that time. The patient was transferred, admitted, and underwent a repeat diagnostic fast during which a 1:1 was present and her parents were asked to remain outside the hospital until completion. She maintained her plasma glucose in the normal range (> 70 mg/dL [3.89 mmol/L]) for the first 19 hours of the fasting test. The fast was terminated at 23 hours for elevated POC BOHB of 3.4 mmol/L, repeat 3.2 mmol/L. Samples obtained at the end of the fast showed plasma glucose 67 mg/dL (3.72 mmol/L), HCO_3 16 mmol/L, lactate 2.1 mmol/L, BOHB 3.59 mmol/L, ammonia 11 µmol/L, insulin 3.8 µIU/mL (26.4 pmol/L), and C-peptide 0.3 ng/mL (0.10 nmol/L) (ratio 0.3).

Table 1. Expected values for critical samples during hypoglycemia in a healthy child

Laboratory test	Patient value	Expected value
HCO_3	18 mmol/L	≥ 18 mmol/L
Lactate	2 mmol/L	0.5-2.1 mmol/L
BOHB	3.59 mmol/L	≥1.8 mmol/L
Cortisol	10.5 µg/dL (290 nmol/L)	≥10 µg/dL (276 nmol/L)
Growth hormone	6.66 ng/mL	≥ 5 ng/mL
IGF-1	94 ng/mL	10-160 ng/mL
Ammonia	33 µmol/L	9-54 µmol/L
Insulin	48.1 µIU/mL (334 pmol/L)	Undetectable
C-peptide	0.2 ng/mL (0.07 nmol/L)	< 0.5 ng/mL (0.17 nmol/L)

Abbreviations: BOHB, β-hydroxybutyrate; IGF1, insulin-like growth factor-1.

Given the level of ketosis was inconsistent with the level of insulinemia, a concern for laboratory assay interference was raised. Additional testing was sent on samples with an insulin assay measured by liquid chromatography–MS (LC-MS) to be compared directly to prior immunoassays sent. As seen in Table 2, the initial insulin levels measured by immunoassay were falsely elevated and the patient was later found to have high HAMA (enzyme-linked immunosorbent assay) at 181 ng/mL. Insulin antibodies were also measured and were negative (< 5 µU/mL).

Outcome and Follow-up

The patient was discharged home with a presumed diagnosis of idiopathic ketotic hypoglycemia. She has had no further episodes of hypoglycemia with more than a year's follow-up since initial presentation. Outpatient laboratory values obtained during a visit demonstrated higher insulin values when measured by immunoassay than when measured by LC-MS/MS both fasting and post meal.

Discussion

Endogenous human heterophilic antibodies are antibodies that are formed by exposure to external antigens and have the ability to bind to immunoglobulins of other species [6]. A common antibody that falls in this category is HAMA—endogenous antibodies that form against murine monoclonal immunoglobulin. The stimulus for the production of these antibodies is generally unknown; however, in a study by Koshida et al [7] the overall prevalence of HAMA in a randomly collected sample population was 11.7%. These circulating heterophile antibodies may interfere with immunoassay measurements as most immunoassay reagents used for hormone measurement use antiserum samples derived from animals [6]. Falsely elevated levels occur when nonspecific HAMAs cross-link isotypic determinants expressed on the Fc portions of the capture and the signal antibodies of "sandwich" immunoassays. As seen in this case, this can lead to forming more "sandwiches" and a falsely elevated signal, the extent of which is generally unknown and unpredictable [8]. Several techniques have been developed to neutralize the effects of HAMA, including the addition of nonimmune mouse immunoglobulin G or heating the specimen in a sodium acetate buffer if the antigen is heat stable. Other testing options include use of LC-MS.

When the clinical picture is inconsistent with the laboratory evaluation, as in the case presented, this should trigger further investigation. Because of the elevated BHOB, a marker with higher sensitivity and specificity for insulin-mediated hypoglycemia, assay interference was suspected as the explanation for the high insulin in this patient. In cases of elevated insulin, it is critical to establish the difference between insulin assay interference and factitious insulin through alternative laboratory methods. In the future, simple procedures to refute or prove HAMA interference could be performed including a dilution

Table 2. Summary of insulin measurements from the case presentation

	Plasma glucose, mg/dL	Plasma insulin by immunoassay, μIU/mL[a] (pmol/L)	Plasma insulin by LC-MS/MS μIU/mL (pmol/L)	C-peptide, ng/mL (pmol/L)	Insulin: C-peptide ratio immunoassay vs LC-MS/MS
Initial fast	51	48.1 (334.05)	—	0.2 (70)	4.77 (no comparison)
Representation	41	42.0 (291.69)	2 (13.89)	0.4 (130)	2.2 vs 0.1
Second fast	67	3.8 (26.39)	2 (13.89)	0.3 (100)	0.3 vs 0.1
AM fasting	80	2.2 (15.28)	< 3 (< 20.84)	0.5 (170)	0.09 vs 0.1
1 h post meal	90	7 (48.62)	4 (27.78)	1.5 (500)	0.1 vs 0.06

Abbreviation: LC-MS/MS, liquid chromatography–tandem mass spectrometry.
[a]SI conversion according to *AMA Manual of Style*, 11th edition.

test of the relevant sample, precipitation of HAMA using a polyethylene glycol addition, or insulin measurement by a different immunoassay. Misdiagnosis could lead to the serious implication of Munchausen by proxy resulting in the removal of a child from their home and parents potentially being charged with a crime. Overall, there is a very low level of suspicion for an underlying hypoglycemia disorder in this patient based on the diagnostic fast demonstrating appropriate fasting adaptation and a lack of further hypoglycemic events during more than a year of follow-up. The underlying cause for the initial presentation is likely idiopathic ketotic hypoglycemia, which is typically seen in toddlers presenting with shortened fasting tolerance with marked ketosis, more frequently during an intercurrent illness, although the triggering event for such a severe presentation in this patient remains unknown.

Learning Points

- Falsely elevated thyrotropin, adrenocorticotropin, follicle-stimulating hormone, parathyroid hormone, insulin-like growth factor-1, prolactin, β-human chorionic gonadotropin, and calcitonin levels due to assay interference caused by heterophile antibodies have been reported previously in the literature.
- This is a report of insulin immunoassay interference due to heterophilic antibodies leading to an erroneous diagnosis.
- Potential assay inference is important to recognize when the clinical presentation and laboratory evaluation are inconsistent.

Contributors

M.C. was the training fellow supervised by K.L. and K.F.L. providing inpatient management during the initial hospitalization; the patient was subsequently followed by D.D.L. for outpatient management. M.C. wrote the first draft of the manuscript and was supervised by D.D.L. All authors contributed to critical manuscript revisions, and read and approved the final submitted version.

Funding

No public or commercial funding.

Disclosures

D.D.L. has received research funding from Zealand Pharma, Tiburio Therapeutics, Twist Bioscience, and Crinetics Pharmaceuticals for studies not included in this manuscript; has received consulting fees from Zealand Pharma, Crinetics Pharmaceuticals, Hanmi Pharmaceutical, and Eiger Biopharmaceuticals not related to this manuscript; and is named inventor in USA patent No. 9,616,108, 2017; USA patent No. 9,821,031, 2017; Europe patent No. EP 2120994, 2018; and Europe patent No. EP2818181, 2019. These patents are not related to the work included in this manuscript. The other authors have nothing to disclose.

Informed Patient Consent for Publication

Signed informed consent could not be obtained from the parents of this patient but has been approved by the treating institution.

Data Availability Statement

All data analyzed during this case report are included in this article. Further enquiries can be directed to the corresponding author.

References

1. Haddad RA, Giacherio D, Barkan AL. Interpretation of common endocrine laboratory tests: technical pitfalls, their mechanisms and practical considerations. *Clin Diabetes Endocrinol*. 2019;5(1):12.
2. Gulbahar O, Konca Degertekin C, Akturk M, *et al*. A case with immunoassay interferences in the measurement of multiple hormones. *J Clin Endocrinol Metab*. 2015;100(6):2147-2153.
3. Laudes M, Frohnert J, Ivanova K, Wandinger KP. PTH immunoassay interference due to human anti-mouse antibodies in a subject with obesity with normal parathyroid function. *J Clin Endocrinol Metab*. 2019;104(12):5840-5842.
4. Giurgea I, Ulinski T, Touati G, *et al*. Factitious hyperinsulinism leading to pancreatectomy: severe forms of Munchausen syndrome by proxy. *Pediatrics*. 2005;116(1):e145-e148.
5. Rabbone I, Galderisi A, Tinti D, Ignaccolo MG, Barbetti F, Cerutti F. Case report: when an induced illness looks like a rare disease. *Pediatrics*. 2015;136(5):e1361-e1365.
6. Tate J, Ward G. Interferences in immunoassay. *Clin Biochem Rev*. 2004;25(2):105-120.
7. Koshida S, Asanuma K, Kuribayashi K, *et al*. Prevalence of human anti-mouse antibodies (HAMAs) in routine examinations. *Clin Chim Acta*. 2010;411(5-6):391-394.
8. Klee GG. Human anti-mouse antibodies. *Arch Pathol Lab Med*. 2000;124(6):921-923.