ADRENOCORTICOTROPIC HORMONE IMMUNOASSAY INTERFERENCE IN A PATIENT WITH SUBCLINICAL HYPERCORTISOLISM

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Abstract

Context. Immunoassays are vulnerable to occasional analytical errors despite their sensitivity and specificity. Adrenocorticotrophic hormone (ACTH) is among hormones which are vulnerable to assay interference.

Objective. In this case report, we aimed to present a case of ACTH interference in a patient with subclinical hypercortisolism.

Case report. We present a case of subclinical hypercortisolism with unexpectedly elevated levels of ACTH in whom interference was shown by different biochemical methods.

It is important for the clinician to keep in mind the possibility of interference when clinical and laboratory results are discordant. The communication between the clinician and laboratory staff is important while interpreting results. This case report shows that ACTH interference should be considered in patients with subclinical hypercortisolism who have unexpectedly high levels of adrenocorticotrophic hormone.

Keywords: Adrenocorticotrophic hormone, ACTH interference, subclinical hypercortisolism.

INTRODUCTION

Immunoassays are vulnerable to occasional analytical errors despite their sensitivity and specificity. Although not frequent, interference in immunoassays may have serious implications in clinical practice. Interference may be caused by the presence of endogenous antibodies, such as heterophilic antibodies, antianimal antibodies or autoantibodies as well as by exogenous antibodies that are given for therapeutic intentions (1, 2). In this case report we aimed to present a case of subclinical hypercortisolism with unexpectedly high levels of adrenocorticotrophic hormone (ACTH).

CASE DESCRIPTION

A 51-year-old female patient was referred to an Endocrinology clinic from a Cardiology department for evaluation of an adrenal mass and hypertension. She had bilateral nodular lesions characteristic of bilateral macronodular hyperplasia found in computer tomography (Fig. 1). She was on treatment with amlodipine/valsartan 10/160 mg for hypertension and was normotensive under this treatment. Her physical examination was not compatible with Cushing syndrome. She was overweight (BMI: 28.5 kg/m²) without Cushingoid features. For evaluation of adrenal mass and hypertension we have initiated hormonal assessments. Overnight dexamethasone suppression test (DST) was unable to suppress cortisol (114.5 nmol/L). Her serum cortisol level after 2-day 2 mg DST was also non-suppressed (96.8 nmol/L). Basal plasma ACTH level was 58.5 pmol/L (0-10.1 pmol/L). Repeated measurements of ACTH were all elevated (55.9 pmol/L, 50.2 pmol/L, 54.3 pmol/L respectively). Her basal serum cortisol level was 260.1 nmol/L and serum cortisol level after 8 mg DST was 103.7 nmol/L. Her 24-hour urinary free cortisol was normal. She was in premenopausal period and her dehydroepiandrosterone sulfate (DHEAS) levels were 56.2 and 53.98 µg/dL (25-460 µg/dL). Her results of hypothalamic-pituitary-adrenal (HPA) axis are shown in Table 1. Hypophysis MRI showed a barely visualized 3 mm pituitary microadenoma. Evaluation for pheochromocytoma and primary hyperaldosteronism as well as adrenocortical carcinoma and metastasis were unremarkable. She was tested for primary hyperaldosteronism with discontinuation of calcium channel blocker and angiotensin receptor blocker agents and replaced with doxazosin for two weeks before screening.

Although she had >50% suppression after 8 mg DST and had a barely visualized hypophyseal

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Figure 1. Abdomen Computer Tomography showing characteristic features of bilateral macronodular hyperplasia.

ACTH (pmol/L)

Figure 2. Results of ACTH levels after serial dilutions showing lack of linearity.

Table 1. Hormonal evaluation regarding ACTH-cortisol axis

	Serum Basal Cortisol (nmol/L)	Serum Cortisol after 1 mg DST (nmol/L)	Midnight serum cortisol (nmol/L)	Serum Cortisol 2-day 2 mg DST (nmol/L)	Serum Cortisol after 8 mg DST (nmol/L)	24-hour urine cortisol (µg/24hours)	Basal plasma ACTH (pmol/L)	DHEAS (µg/dL)
Reference and normal levels	138- 635	<50	<206.93	<50	N/A	20.9- 292.3	0-10.1	25-460
Levels for the patient	260.1	114.5	142.1	96.8	103.7	155	58.5 55.9 50.2 54.3	56.2 53.98

ACTH: Adrenocorticotrophic hormone, DST: Dexamethasone suppression test, N/A: non-applicable.

Table 2. Results of interference tests

	Measurement on a different analytical platform*	Measurement after PEG precipitation**
Plasma ACTH levels (pmol/L)	1.2	<5

ACTH: Adrenocorticotrophic hormone, PEG: polyethylene glycol.

* Samples were tested on a solid-phase, two site electrochemiluminescence immunoassay platform (analytical range; 0.2-440 pmol/L; Elecsys E170; Roche Diagnostics) **Precipitation was performed with PEG 6000 solution to remove interfering antibodies and plasma ACTH levels were measured in supernatants (analytical range; 1.1-333 pmol/L, Immulite 2000 XPi, Siemens Healthcare diagnostics).

microadenoma with elevated ACTH levels, she did not have Cushingoid features, had modest elevation in cortisol levels (after DST), normal 24-hour urinary free cortisol, DHEAS levels in the lower limit and had no osteoporosis in bone mineral density. When all these results of clinical and biochemical incompatibility are taken together; before performing further invasive tests such as corticotropin releasing hormone/desmopressin (CRH/DDVAP) stimulation test in combination with a separate DST or inferior petrosal sinus sampling (IPSS), the possibility of ACTH interference came forward.

ACTH measurements in our center were performed by a solid phase, two-site enzyme chemiluminescent system (analytical range: 1.1-333 pmol/L, Immulite 2000 XPi, Siemens Healthcare Diagnostics) and all ACTH values were elevated. We considered the possibility of interference, due to unexpectedly high ACTH levels, which was inconsistent with the clinical findings. In order to evaluate assay interference, measurement on a different analytical platform was performed. Samples were tested on a solid-phase, two site electrochemiluminescence immunoassay platform (analytical range: 02-440 pmol/L; Elecsys E170; Roche Diagnostics) and her ACTH level was 1.2 pmol/L (1.58-13.93 pmol/L) (Table 2). Phosphate-buffered saline-PEG 6000 (Merck, Germany) precipitation was performed with PEG 6000 solution to remove interfering antibodies and plasma ACTH levels were measured in supernatants. Post-PEG ACTH concentration was undetectable

(Table 2). Finally, plasma ACTH levels were measured after serial dilutions (1/2, 1/4, 1/8, 1/16, 1/32) to check for lack of linearity and recovery (parallelism) (Fig. 2). ACTH measurements after serial dilutions and PEG precipitation were performed by the same solid phase chemiluminescent system (analytical range; 1.1-333 pmol/L, Immulite 2000 XPi, Siemens Healthcare Diagnostics). Also, other hormonal assessments which are vulnerable to immunoassay interference were performed, such as thyroid stimulating hormone (TSH), free thyroid hormones, Beta-human chorionic gonadotropin (b-HCG), follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), parathyroid hormone (PTH) and thyroid autoantibodies using chemiluminescence immunoassay platform (Immulite 2000 XPi, Siemens Healthcare Diagnostics). All values were in normal range. Also rheumatoid factor (RF) level was measured as it is one of the most common interfering antibodies (3). Her RF level was increased (>120 IU/mL (reference range: <4 IU/mL)), which was analyzed using AU5800 platform (Beckman Coulter). She is now being treated in a rheumatology clinic for rheumatoid arthritis with decreased symptoms after treatment. In her 3 years of follow-up she did not develop any Cushingoid features, her metabolic parameters, BMI and waist circumference are stable and her cortisol levels after overnight DST are still in subclinical range in her yearly follow-up. Her control MRI of adrenals showed stable findings and genetic testing for Armadillo repeat-containing protein 5 (ARMC5) mutation was negative.

DISCUSSION

In this case report, ACTH interference was indicated by three different methods used for evaluation of assay interference such as: using another analytical platform, PEG precipitation and serial dilutions. Use of a different analytical platform to rule out interference is recommended as interference usually involves a specific antibody or a set of antibodies (4). Shi *et al.* (5) have recently published an article regarding a new method to solve the clinically discrepant results. In their new method they suggest performing a liquid chromatography- tandem mass spectrometry (LC-MS/MS) to measure biologically active intact ACTH which is a promising and highly selective method.

Assay interference may be detected in multiple hormones or it may be limited to one hormone assay. Gulbahar *et al.* (2) reported assay interference in multiple hormone analyses as well as ACTH

interference in their case report. In our previous research we have shown antibody interference for ACTH in 4 patients with adrenal adenoma, who had a combination of non-suppressed ACTH levels and non-suppressed cortisol levels after 1 mg DST, in whom clinical management was affected by inaccurate results (6). Plasma ACTH concentration is important in patients with adrenal incidentaloma who have mildly increased cortisol levels after 1 mg DST. It is also important to show ACTH independency when adrenalectomy is considered. Thus, high variability and unexpected ACTH levels due to analytical errors may complicate interpretation of the clinical situation. Invitti *et al.* (7) showed in their multicenter study that, among patients in whom ACTH-independent Cushing syndrome is considered, plasma ACTH concentrations were detectable in 58% and were in the normal range in 28%. Analytic variability in ACTH assay is more frequent with low ACTH concentrations (8).

In this case report, RF may be the possible reason for ACTH interference, however high levels of RF do not rule out interference caused by other antibodies. RF is generally an immunoglobulin M (IgM) antibody having affinity to the Fc region of the patient's own IgG antibody (9). Depending on the platform used, RF may be positive in 5-10% of general population and in 70% of patients with diagnosis of rheumatoid arthritis (9). In our previous study (6), among 4 patients with ACTH interference, RF was possibly responsible for interference in one patient.

Hypophysis MRI was unnecessary for this patient. The average frequency of pituitary adenoma was found to be 10.6% in a study investigating autopsy series (10). This MRI showing pituitary microadenoma is an incidental finding. Inferior petrosal sinus sampling (IPSS) would be even more unnecessary for evaluation of this patient. Thus, before planning invasive tests, when there is clinical and biochemical discrepancy, interference should be ruled out.

As autonomous cortisol secretion is also known to be associated with bilateral macronodular adrenal hyperplasia and is an infrequent cause of ACTH-independent Cushing syndrome, investigation for aberrant receptors could be done under suppression of 1 mg dexamethasone in order to avoid any effect of ACTH on steroidogenesis (11).

Pre-analytical practices such as inappropriate handling of the specimens is also another cause of false results when ACTH measurement is considered. Maintenance of samples in the room temperature, delayed centrifugation and separation of plasma,

hemolysis and excessive use of EDTA were shown to cause a false decrease in ACTH concentration (12). All measurements in our case were made by paying extra attention to these important issues.

Prolonged hypercortisolism is associated with increased mortality and morbidity, however no agreement exists regarding diagnosis and treatment of subclinical hypercortisolism (13, 14). Modern two-site sandwich immunometric assay use for measurement of ACTH levels is a subject for analytical interference leading to false positive or negative results. Therefore, alternative ACTH assays should be used for diagnosis and differential diagnosis of disorders regarding HPA axis (12, 15, 16). We have shown that ACTH interference should be kept in mind in patients with incompatible clinical and laboratory results.

In conclusion, it is important for the clinician to keep in mind the possibility of interference when clinical and laboratory results are discordant. The communication between clinician and laboratory staff is important while interpreting results.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This research did not receive any specific grant from agencies in the public, commercial or not-for-profit sectors.

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