Laboratory Case Report

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False high testosterone of unknown reason in a clinically inconspicuous female

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Abstract

Background: This report investigates an unusual total testosterone result in a middle-aged female.
Case presentation: A 40-year-old female patient was found to have high serum total testosterone of 30.82 nmol/L on a Beckman Coulter UniCel® DxI800 automatic chemiluminescence immunoanalyzer without appropriate clinical appearance. Heterophile antibody interference was considered for elevated testosterone and investigated; reanalysis of the original serum sample and liquid chromatography with tandem mass spectrometry (LC-MS/MS) were performed. Reanalysis of the original serum sample using Roche® and Siemens® immunoassays both gave a normal total testosterone level. Beckman Coulter® also excluded the possibility of interference by heterophile antibodies. Finally, LC-MS/MS showed that the total testosterone level was in the normal range.
Conclusions: This report highlights the importance of ruling out the interfering factors for false high values of the analyte in laboratories.

Keywords: interference; misdiagnosis; total testosterone.

Introduction

Testosterone is the principal androgen hormone, produced mainly by the Leydig cells in males. Testosterone in females is produced mainly by the ovaries and the adrenal gland. Testosterone results from the peripheral conversion of androstendion produced in the theca cells of the ovary at very small concentrations. In the case of hyperandrogenemia, the theca cell production of testosterone is increased. The other source of small amounts of testosterone is the production of dehydroepiandrosterone (DHEA) in the adrenal cortex. About 60% of testosterone in the blood binds with testosterone globulin, 38% combines with albumin and 2% is free. Total testosterone levels are routinely assessed by the automated Access Testosterone assay, a competitive binding immunometric assay performed using the Unicel® DxI 800 (Beckman Coulter, Brea, CA, USA) platform. However, these tests are prone to interference by proteins in the patient's blood (such as heterophile antibodies and binding proteins) and androgenic compounds. These interfering substances can also be present in men, but they seldom affect the results because of higher testosterone concentrations. There is a dearth of data regarding falsely elevated testosterone levels due to heterophile antibodies in women [1]. Studies relating to androgenic compound interference are also sparse. Examples include Danazol and mifepristone [2]. Sex hormone binding globulin (SHBG) can also cause spuriously elevated testosterone levels in women [3, 4]. Falsely elevated testosterone may lead to a misdiagnosis, which may, in turn, result in treatments with severe side effects. Furthermore, testosterone levels are difficult to determine because of diurnal variations, poorly standardized reference ranges and the inability of immunoassays to detect low concentrations accurately; sometimes, falsely elevated test results may be solely based on the assay's limitations [5, 6]. Our case highlights the importance of excluding assay interference in females without appropriate clinical appearance but deviant testosterone levels.

Case presentation

A 40-year-old female patient was referred to an endocrine clinic for clinical evaluation of a markedly elevated serum total testosterone level of 30.82 nmol/L (normal range 0.35–2.6 μmol/L). The patient complained about irregular menstruation and alopecia but denied a reduction

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in breast size, deepening of voice or excess body hair. She was on a diet by reducing intake of food at the time. She had a total weight loss of 13.5 kg in the past 6 months. She denied smoking, drinking alcohol or using illicit drugs. In the past, she was not treated with immunoglobulins or antibodies for therapeutic or diagnostic reasons.

A physical examination showed that she was a well-developed woman without any signs of reduction in breast size, acne, cliteromegaly and hirsutism. Her physician had a suspicion of polycystic ovarian syndrome or follicular cell proliferation syndrome. There were no palpable adnexal and adrenal masses. Her physician had ordered a total testosterone level along with follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), progesterone (Prog), estradiol (E2), DHEA, SHBG and growth hormone (GH) levels. The laboratory tests resulted in normal hormone values except an elevated total testosterone level. All other laboratory tests were normal. Other investigations were also normal, including routine biochemistry (e.g. albumin, total protein, etc.), protein electrophoresis, a full blood count, thyroid function, tumor markers, serum osteocalcin, parathyroid hormone, plasma cortisol and adrenal cortical hormone (ACTH).

Two months earlier, her total testosterone level was 29.13 nmol/L. Repeat laboratory tests demonstrated a total testosterone level of 22.40 nmol/L on a Beckman Coulter UniCel® DxI800 automatic chemiluminescence immunoanalyzer in another hospital about 15 days previously.

At our laboratory, it is a routine practice to use different detection systems to reanalyze samples with abnormal results.

Measurements with the Roche cobas e601 Elecsys® (Roche Diagnostics, Mannheim, Germany) immunoassay in two other hospitals gave normal testosterone values of 0.38 mmol/L and 0.66 nmol/L, respectively. When measured in a third hospital with the Siemens ADVIA Centaur® XP (Siemens, Munich, Germany) automated chemiluminescense immunoassay, the total testosterone was also in the normal range of 1.08 nmol/L.

Due to the suspected interference, we sent the sample to the Complaint Handling Unit of Beckman Coulter® for testing. The measurement of the neat sample confirmed the high values as determined using the Beckman system before. The sample was pretreated using heterophile blockers and the blockers of alkaline phosphatase, but these pretreatment modalities did not reduce the signal. A patient-related source of interference as a cause for the erroneously high testosterone results was not found for this patient. Therefore, the sample was finally sent to a reference laboratory where testosterone was measured using liquid chromatography with tandem mass spectrometry (LC-MS/MS), thus confirming a testosterone value of 0.75 nmol/L, consistent with her clinical presentation. The patient had a normal total testosterone level and the result was consistent with her clinical presentation.

Ethical approval

The research related to human use has been complied with all the relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration, and has been approved by the First Affiliated Hospital of Nanjing Medical University.

Discussion

In women, commercial immunoassays cannot be calibrated accurately because of the lack of normal testosterone controls at minimal concentrations [5–7]. Commercial immunoassays consist of several antibody reactions and are based on the assumption that each antibody has a strong and specific affinity for a unique antigen, which is not the case. Therefore, testosterone measurements based on commercial immunoassays may interfere with other substances. So far, mass spectrometry has proved to be the best tool for measuring testosterone because of its high accuracy. However, both direct immunoassay and mass spectrometry need to operate within a quality framework and be actively engaged in external quality control processes and standardization to ensure appropriate interpretation, irrespective of the particular laboratory [8].

Different studies have investigated the possible causes of interference in serum testosterone measurement in women. Testosterone assays could be interfered by either endogenous compounds or drugs, which have a high degree of structural similarity to testosterone. Middle [9] discussed that DHEA, structurally similar to testosterone, might cross-react with testosterone measurements due to its relatively high circulating concentrations. Maca (*Lepidium meyenii*), a plant product, was suspected to cause testosterone immunoassay interference in females [10]. There are similar reports, such as studies on danazol (a gonadotrophin inhibitor with androgenic and antioestrogenic properties) and mifepristone [2]. Besides, Raff and Sluss [11] recommended that great attention should be paid to pre-analytical issues for testosterone assays. But in our case, the patient had a normal DHEA level and did not have any history of drug ingestion of this kind. We

are therefore still uncertain about the cause of the falsely elevated testosterone level in our patient.

Conclusions

Our case suggests the importance of clinical judgment in interpreting unexpected laboratory results. For falsely elevated testosterone levels, it is better to reanalyze samples with different detection systems. If the results vary in different systems, it is recommended to use LC-MS/MS to rule out assay interference and confirm the testosterone level.

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