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Case report

A diagnostic curiosity of isolated androstenedione elevation due to autoantibodies against horseradish peroxidase label of the immunoassay

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ABSTRACT

Two sisters with hirsutism presented with mild hirsutism and isolated, grossly elevated (> 34.9 nmol/L) serum concentrations of androstenedione measured by competitive, homogeneous immunoassay. The clinically discordant laboratory results prompted us to look for assay interference. In this immunoassay, horseradish peroxidase (HRP)-conjugated androstenedione competes with endogenous androstenedione for binding with the solid-phase polyclonal rabbit IgG antibodies. After a wash step, the amount of signal generated by the bound HRP conjugate is inversely proportional to the androstenedione concentration. Alternative analysis by tandem mass spectrometry (a good first line option for troubleshooting) and repeating the competitive immunoassay after polyethylene glycol treatment returned androstenedione concentrations within reference limits. These findings suggested that the original result was spuriously elevated due to assay interference. Additionally, the patient samples were pre-incubated with heterophile blocking reagents, normal rabbit IgG antibodies and HRP-conjugated normal goat IgG antibodies, followed by repeat measurement using the immunoassay. Only samples pre-incubated with HRP-conjugate returned significantly lower androstenedione (9.5 and 12.5 nmol/L, respectively), implying neutralisation of the interfering antibodies. Androstenedione remained grossly elevated in the other experiments. This deductive exercise showed that the interference is due to autoantibodies against the HRP label used in the immunoassay. Another immunoassay using HRP label (5α-dihydrotestosterone) also produced gross elevation that was normal by tandem mass spectrometry analysis. Assay interferences, though not uncommon, are frequently overlooked. Laboratory results discordant with clinical features should prompt consideration of assay interference to avoid unnecessary investigations and treatment. This is the first report of autoantibodies against the HRP label used in immunoassay.

1. Introduction

Hirsutism is a condition of male-pattern hair growth in women, and may be idiopathic or secondary to hyperandrogenism [1,2]. While polycystic ovarian syndrome is the most common cause of hyperandrogenism, other diagnoses such as non-classical congenital adrenal hyperplasia and androgen-secreting tumours must be considered [1]. We describe two female siblings with mild hirsutism and gross elevation of androstenedione in isolation.

2. Materials and methods

2.1. Clinical presentation

A 14-year-old girl of Chinese-Japanese descent presented with hirsutism for the last 4 years. She was otherwise well with no past medical history. Menarche was at 12 years of age, and she had regular menses. Her height was 158 cm (75th percentile), weight 40.5 kg (25–50th percentile) and body mass index 16.2 kg/m^2 (10th percentile). She had increased body hair affecting the upper lip, forearms and upper back

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Table 1
Details of selected investigations for the proband and her sister. The upper limit of measurement range of the androstenedione immunoassay is 34.9.

Laboratory investigation	Reference intervals	Proband	Younger sister
Androstenedione by immunoassay, nmol/L	1.4–9.7	> 34.9	> 34.9
Androstenedione post-PEG, nmol/L	Not available	4.97	2.72
Androstenedione by tandem mass spectrometry, nmol/L	2.8–8.4	3.87	2.09
17-hydroxyprogesterone, nmol/L	0.1–2.2	12.9	3.1
Dehydroepiandrosterone sulfate, μmol/L	1.48–6.92	3.57	2.08
Testosterone, nmol/L	0.22–2.9	1.68	0.45
Dihydrotestosterone by immunoassay, nmol/L	0.08–1.25	5.83	8.87
Dihydrotestosterone by tandem mass spectrometry, nmol/L	< 1.03	0.21	0.31
Androstenedione post-rabbit IgG incubation, nmol/L	1.4–9.7	> 34.9	> 34.9
Androstenedione post-goat IgG with horseradish peroxidase label incubation, nmol/L	1.4–9.7	9.5	12.5

with a Ferriman-Gallway score of 8 (mild hirsutism) [1]. There was no deepening of the voice or clitoromegaly. Breast and pubic hair development were at Tanner stage 5. Blood pressure was normal. Written inform consent from the parents of the siblings were obtained for publication of this case report.

Her younger sister, age 12 years, also had mild hirsutism. Her height was 151.9 cm (50–75th centiles), weight 35.1 kg (25–50th centile) and her body mass index 15.2 kg/m^2 (5th centile). Menarche occurred at 11 years and her menses were regular. There was no deepening of the voice or clitoromegaly.

The proband initially presented with isolated elevation of androstenedione at 23.5 nmol/L (reference intervals: 1.4–9.7) with normal testosterone (measured using chemiluminescence immunoassay on DxI 800 platform, Beckman Coulter, Inc., Fullerton, CA), dehydroepiandrosterone sulfate (DHEA-S, measured using chemiluminescence immunoassay on Immulite 2000 platform, Siemens Healthcare, Hamburg, Germany) and 17-hydroxyprogesterone concentrations (radioimmunoassay, Tecan Co., Salzburg, Austria) (Table 1). Serial monitoring of her serum androstenedione 7 months and 12 months later showed persistent and isolated gross elevation (> 34.9 nmol/L).

Ultrasound scan and magnetic resonance imaging of her pelvis and abdomen did not show any adrenal or ovarian tumour, and there were no features suggestive of polycystic ovaries. Her karyotype was normal female 46XX. For the younger sister, laboratory investigations showed that she also had isolated grossly elevated androstenedione at > 34.9 nmol/L, with normal testosterone, DHEA-S and 17-hydroxyprogesterone.

2.2. Androstenedione immunoassay

The androstenedione was measured using a competitive, homogeneous immunoassay (IBL, Hamburg, Germany). This immunoassay uses horseradish peroxidase (HRP)-conjugated androstenedione to compete with endogenous androstenedione for binding with the solid-phase polyclonal rabbit IgG antibodies (Fig. 1).

2.3. Measurement of androstenedione by mass spectrometry

Method comparison studies are commonly used to investigate assay interference [3,4]. A tandem mass spectrometry method performed at Mayo Medical Laboratories (Rochester, USA) was chosen for these cases as the physical principle of the assay is completely different than that of immunoassay.

2.4. Polyethylene glycol (PEG) precipitation

Polyethylene glycol (PEG) is a chemical that nonspecifically precipitates large molecules (including immunoglobulins). Equal volumes of serum samples of the patients were mixed with PEG 6000 (25% (w/v), Merck, Darmstadt, Germany; reference 817,007) and agitated for 30 s. After the mixture was allowed to stand for 5 min, it was centrifuged at 3000*g* for 5 min. Androstenedione was measured immediately on the supernatant of the sample after centrifugation.

2.5. Heterophile blocking tube incubation

To investigate whether the heterophile antibodies may explain the clinically discrepent laboratory result, 500 μL of each patient sample was added into the heterophile blocking tube (Scantibodies Laboratory Inc., Santee, CA, USA) and mixed. Following 1 h of incubation, the samples were recentrifuged and remeasured, according to instructions by the manufacturer.

2.6. 5α-dihydrotestosterone immunoassay

To confirm the components of the immunoassay were affected, serum samples from both sisters were analysed on a competitive 5α-dihydrotestosterone (DHT) immunoassay (IBL), which also employed solid-phase rabbit IgG antibodies and HRP as conjugate label.

2.7. Pre-incubation with rabbit antibodies and horseradish peroxidase

To identify the component of the immunoassays that were affected by the antibody interference, the serum samples from both sisters were separately pre-incubation with normal rabbit IgG (sc-3888, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and normal goat IgG with HRP conjugate (sc-2741, Santa Cruz Biotechnology). These pre-incubation steps were designed to neutralize interfering antibodies against rabbit IgG and HRP, respectively.

3. Results

3.1. Mass spectrometry

Measurements using tandem mass spectrometry found androstenedione concentrations to be within the reference interval in both siblings (Table 1).

3.2. Polyethylene glycol precipitation

Pre-treatment of patient serum with PEG normalised the androstenedione concentrations (Table 1). This finding is consistent with assay interference from antibodies.

3.3. Heterophile blocking tube incubation

Incubation of the serum samples with heterophile blocking tube did not reduce the grossly elevated androstenedione concentration (> 34.9 nmol/L).

3.4. 5α-dihydrotestosterone immunoassay

The DHT concentrations for both sisters were grossly elevated by the immunoassay but were within reference interval by tandem mass spectrometry (Table 1).

3.5. Pre-incubation with rabbit antibodies and horseradish peroxidase

Pre-incubation of their samples with normal rabbit IgG did not reduce the grossly elevated androstenedione (Table 1). Conversely, pre-