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Falsely Elevated Testosterone Levels: Role of LC-MS/MS

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Abstract

• Immunoassays are commonly used in clinical laboratories for analyzing protein antigens and steroid hormones, including testosterone

• Advantages: availability as commercial kits, low cost, simplicity

• When there is discordance between clinical scenario and laboratory tests consider laboratory errors, including interfering substances

Case Report

- A 17-year-old female with facial acne was referred to us for elevated testosterone levels
- She was of Greek descent and reported having had coarse dark hair growth on upper lip, chin, chest for which she previously underwent laser therapy
- Her menstrual cycles were regular and predictable
- On physical examination, she was normotensive with BMI of 22kg/m^2. She had coarse dark hair on upper abdomen, lower abdomen and medial thighs with Ferriman-Gallwey score of 7, genital exam did not reveal clitoromegaly
- On initial testing, serum total testosterone was 417ng/dL (range: <75ng/dL)

Laboratory Values

Initial test results:

		4/12/2018 0844	4/16/2018 0815	4/20/2018 1301
ENDOCRINOLOGY				
Androstenedione	Latest Range: 22 - 225 ng/dL			144 *
Calc Bioav. Testos...	Latest Range: 1.5 - 43.4 ng/dL		147.7 ▲	156.2 ▲
Calc Free Testoste...	Latest Range: <1.9 ng/dL		6.3 ▲	6.7 ▲
Estradiol	Latest Units: pg/mL			50 *
Free Androgen Index	Latest Range: 0.7 - 10.9 %		29.9 ▲	29.8 ▲
FSH	Latest Units: mIU/mL			4.7 *
Insulin	Latest Range: <14 uIU/mL	8		
LH	Latest Units: mIU/mL			6.5 *
17-Hydroxyprogeste...	Latest Range: 35 - 413 ng/dL	71 *		
Progesterone	Latest Units: ng/mL	1.1 *		
Prolactin	Latest Range: <27.0 ng/mL	4.8		
Testosterone Evalu...	No range found		Free and Bioav... *	Free and Bioav... *
Testosterone, total	Latest Range: <75 ng/dL	417 ▲	369 ▲	422 ▲
Sex Hormone Bindin...	Latest Range: 18.2 - 135.5 nmol/L		42.8	49.1

Imaging Tests

- Pelvic and renal ultrasound: normal appearing ovaries, kidneys and adrenal glands
- MRI requested, not done prior to endocrine evaluation

Clinical Course

- Suspected falsely elevated testosterone levels since clinical scenario was discordant from laboratory test results
- On dilution, results were non-linear indicating interference
- Total testosterone levels after addition of heterophilic antibody blocking reagent resulted as 114ng/dL (from 422ng/dL) which indicated heterophile antibody interference.
- To obtain true testosterone levels, we repeated the test using liquid chromatography tandem-mass spectrometry (LC-MS/MS) which revealed total testosterone level as **19ng/dL**
- Pending MRI was canceled

Heterophilic Antibodies: When to suspect interference?

Lab results discordant from clinical picture

- Patients with history of previous false results in immunometric assays
- Patients previously exposed to animal antibodies similar to assay antibodies
- Patients with seropositive rheumatic disease
- When results strongly impact treatment e.g tumor marker hCG

Discussion

• Immunoassays, although widely used, are limited by cross-reactivity, matrix effects and heterophilic antibodies

• LC-MS/MS has superior performance and sensitivity for measurements at low concentrations including steroid hormones such as testosterone, estradiol, cortisol, 17-hydroxyprogesterone

•Repeat testing using LC-MS/MS for steroid hormones, such as testosterone, should be considered to prevent unnecessary repeat testing and imaging when clinical picture and laboratory tests are discordant

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