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Reminder of important clinical lesson

First case report of testosterone assay-interference in a female taking maca *(Lepidium meyenii)*

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Summary

A young female with prolonged intermenstrual bleeding was found to have raised total plasma testosterone of 25.8 nmol/l (NR<2.9 nmol/l) using the Roche Elecsys Testosterone I immunoassay without clinical features of virulisation. Few months ago investigations for lethargy and low libido had shown normal total testosterone of 0.8 nmol/l. Further history revealed that she was using maca extract to improve her lethargy and low libido. Maca is traditionally used for its aphrodisiac and fertility-enhancing properties. Maca use has not been shown to affect serum testosterone in mice and human studies.

Immunoassay interference with maca was suspected. Testosterone immunoassays use monoclonal antibodies specifically directed against testosterone. They are prone to interference from androgenic compounds. Reanalysis of the original serum sample using Elecsys Testosterone II assay, a higher affinity assay, revealed a total testosterone level of 2.9 nmol/l. It is important to exclude assay interference when testosterone level is greater than 5 nmol/l without supportive clinical signs.

BACKGROUND

Most healthcare centres in the UK process testosterone samples using automated assay platforms consisting of direct immunoassays. These are prone to interference from a number of androgenic compounds resulting in falsely elevated testosterone levels in females. Examples include Danazol (a gonadotrophin inhibitor with androgenic and antioestrogenic properties) and mifepristone.[1] Studies relating specifically to assay interference in women are sparse. One study evaluated the difference in serum testosterone results measured by a direct immunoassay and after sample extraction into diethyl ether in 1271 female samples with initial (direct) testosterone concentration of >3.0 nmol/l.[2] The median difference (direct – extracted result) was 1.4 nmol/l (range: 1.2–33.7). The implication of this result is that the substance causing the increase in measured testosterone in the direct assay, is present to a greater or lesser extent in all samples from women with a serum testosterone >3.0 nmol/l. Our case highlights the importance of ruling out assay interference when testosterone levels >5 nmol/l are found without clinical features of hyperandrogenism in females. Furthermore, our case is the first report to date of maca causing testosterone immunoassay interference.

CASE PRESENTATION

A Caucasian female in her thirties was referred to the endocrine clinic by her general practitioner for assessment of a markedly elevated total plasma testosterone of 25.8 nmol/l (NR 0–2.9) discovered when she presented with prolonged intermenstrual bleeding.

She had been investigated by the endocrine unit a few months earlier for symptoms of lethargy, low libido and generalised pains. She was menstruating regularly at the time, and was not taking any medication apart from multivitamins and minerals. In the past, she had suffered from bulimia nervosa from which she recovered 2 years ago. She was a non-smoker, social alcohol user and denied exogenous steroid use. Previous investigations were normal, including a full blood count, routine biochemistry, total plasma testosterone (0.8 nmol/l), thyroid function, anterior pituitary profile and cortisol response to 250 mcg of synacthen. The only abnormality was a mildly raised sex hormone binding globulin (SHBG) of 113 nmol/l (NR 26–110).

Following discharge from our clinic she started taking one teaspoon of maca powder dissolved in milk once daily to improve her energy levels and libido. Within a few weeks of starting maca, she experienced prolonged intermenstrual bleeding. She was taking maca when the blood test showing very high testosterone was performed. She stopped maca 1 month later and had noticed some improvement in bleeding.

On examination, she was of normal body build, normotensive, anxious but well. She had Tanner stage 5 female secondary sexual characteristics without any clinical features suggestive of hyperandrogenism such as acne, hirsutism, frontal scalp cession or clitoromegaly.

INVESTIGATIONS

Lack of clinical manifestations with such high testosterone in a female and a previously normal testosterone raised the suspicion of immunoassay interference. The original high testosterone was obtained using the Roche Elecsys Testosterone I immunoassay (RET I). At our institution, it is routine practice to reanalyse samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS) when assay interference is suspected. However, Roche

had recently released the Elecsys Testosterone II (RET II), reportedly with greater specificity. The original serum sample was therefore retrieved and reanalysed using RET II assay giving a total plasma testosterone level of 2.9 nmol/l (NR 0–1.8). Unfortunately there was not sufficient serum remaining for additional comparison with LC-MS/MS.

On the day of her visit to the endocrine unit 1 month after stopping maca, a repeat total plasma testosterone measured using RET I was 1.5 nmol with an SHBG of 110 nmol/l, confirming assay interference from maca.

OUTCOME AND FOLLOW-UP

The patient was reassured and advised to avoid taking maca.

DISCUSSION

Direct testosterone immunoassay

The RET I immunoassay is based on a competitive test principle using a monoclonal antibody specifically directed against testosterone. Endogenous testosterone released from the sample by 8-anilino-1-naphthalene sulphonic acid and norgestrel competes with the added testosterone-derivative labelled with ruthenium complex for the binding sites on the biotinylated antibody. Between March and May 2010, RET I was upgraded nationally to RET II, which employs a new higher affinity sheep monoclonal testosterone antibody. The new assay has been shown to provide greater accuracy (in the range of 0.025–1.5 ng/ml) due to improved testosterone recovery[3] and less dehydroepiandrosterone sulfate (DHEAS) -cross reactivity and matrix effects with female samples.[4] However, in our patient, the repeat testosterone result with the RET II assay, although 10 times lower than the false result with RET I, was still significantly higher than the testosterone values obtained during the first outpatient visit (before maca use) and the last outpatient visit (by which time she had stopped maca). This implies that RET II assay is still susceptible to a small degree of assay interference.

Maca

Maca is the edible root of the *Lepidium meyenii,* a cruciferous plant (Brassicaceae family) which is cultivated exclusively at an altitude of 4000–4500 m in the Peruvian Central Andes. It is available in different colours (white, yellow, red and black), traditionally used for aphrodisiac and fertility-enhancing properties in males and females as well as for improving menopausal symptoms. Studies in rats have shown that black maca improves sperm production, sperm motility and semen volume while red maca can reduce prostate size in prostate hyperplasia induced by testosterone enanthate. The exact mechanisms of action remain unknown. To date maca has not been shown to affect total serum testosterone levels in humans. A randomised, placebo-controlled study in Peru involving 56 healthy human male subjects showed that maca use did not result in testosterone or gonadotrophin changes.[5] In this study testosterone was measured by radioimmunoassay, which is less susceptible to assay interference. Studies in rats have also shown a lack of effect on testicular testosterone with maca treatment.[6] Our case of assay interference suggests that maca contains a compound with similar moiety to the human testosterone molecule. Maca may be exerting its androgenic effects through actions at the testosterone receptor on target organs without affecting level of testosterone or gonadotrophins.

Learning points

- Testosterone immunoassays used in most healthcare centres use direct immunoassays which are prone to interference from androgenic compounds resulting in falsely elevated testosterone levels in females.
- Maca is a plant product which may cause testosterone immunoassay interference in a female.
- RET II assay employs a new higher affinity sheep monoclonal testosterone antibody. The new assay has been shown to provide greater accuracy in females due to less DHEAS-cross reactivity and matrix effects. However, as our case demonstrates RET II is not entirely free of assay interference.
- High concentrations of testosterone in females (>5 nmol/l) should be interpreted with caution when clinical signs are discordant. Detailed history of over-the-counter products used and repeat analysis of samples using alternative assays or LC-MS/MS should be considered to rule out assay interference before patients are subjected to further investigations.

Competing interests None.

Patient consent Obtained.

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