Spuriously high androstendione concentrations due to assay interference as a cause of diagnostic conundrum in women with oligomenorrhoea

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Abstract

Polycystic ovary syndrome (PCOS) is a diagnosis of exclusion. We present two cases of women with oligomenorrhoea and high concentration of androstendione, suggestive of possible androgen-secreting tumour, caused by assay interference. The first patient, investigated for oligomenorrhoea, had no significant hirsutism or acne. Androstendione concentration was above 10.0 ng/ml (rr: 0.3-3.3 ng/ml). In order to rule out possible androgen-secreting tumour or hypercortisolaemia we performed 48-hour low dose dexamethasone suppression test (LDDST). This failed to demonstrate adequate suppression of androstendione (6.05 ng/ml and 9.32 ng/ml after the first and the second day, respectively). Pelvic ultrasound examination showed polycystic ovaries, while abdominal CT scan failed to show any ovarian or adrenal lesion. Despite such high androstendione concentrations, urinary steroid profile (gas chromatography/mass spectrometry method) yielded normal results. Hence a possibility of androstendione assay interference was raised.

The second patient was also admitted for investigations of oligomenorrhoea. Clinical examination was unremarkable. There was a high concentration of testosterone 0.78 ng/ml (rr: 0.084-0.481 ng/ml) and androstendione above 10.0 ng/ml (rr: 0.3-3.3 ng/ml). LDDST failed to demonstrate any suppression of androstendione, while recalculated concentrations of androstendione after serial dilutions were markedly lower in comparison to initial values. Therefore, such high androstendione concentrations (i.e. above the upper limit of the assay) must have resulted from assay interference. In both cases a final diagnosis of PCOS was established.

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W streszczeniu

W diagnostyce różnicowej zespołu policystycznych jajników (PCOS) należy wykluczyć pozostałe przyczyny hiperandrogenizacji. W pracy przedstawiono przypadki dwóch kobiet z zaburzeniami miesiączkowania i wysokimi wartościami stężeń androstendionu, to jest w zakresie stężeń sugerujących możliwość obecności hormonalnie czynnego guza wydzielającego androgeny. W pierwszym przypadku pacjentki bez klinicznych cech hiperandrogenizacji odnotowano stężenie androstendionu powyżej 10,0 ng/ml (norma: 0,3-3,3 ng/ml). W celu wykluczenia obecności guza hormonalnie czynnego przeprowadzono 48-godzinny test harmonowania z deksametazonem (0,5 mg co sześć godzin), w którym nie stwierdzono zadowalającego spadku stężeń androstendionu (wartości 6,05 ng/ml oraz 9,32 ng/ml, odpowiednio po pierwszym i po drugim dniu testu). W wykonanym badaniu TK jamy brzusznej nie stwierdzono patologii nadnerczy, zaś w badaniu ultrasonograficznym opisano morfologię jajników policystycznych. W związku z powyższym zlecone oznaczenie dobowego profilu steroidowego moczu metodą chromatografii gazowej i spektrometrii masowej. W ocenie dobowego profilu steroidów nie oznaczały nieprawidłowości, co wskazuje na możliwość interferencji w oznaczeniu androstendionu.

Podobnie w drugim opisywanym przypadku wysokiego stężenia testosteronu (0,78 ng/ml przy normie: 0,084-0,481 ng/ml) i androstendionu powyżej 10,0 ng/ml (norma: 0,3-3,3 ng/ml) nie towarzyszyły kliniczne cechy hiperandrogenizacji. W przeprowadzonym 48-godzinnym teście harmonowania z deksametazonem nie stwierdzono spadku stężenia androstendionu. Dodatkowo wartości stężeń androstendionu uzyskane po rekultakcji wartości uzyskanych po rozcieńczeniach były znacznie niższe od wartości wyjściowych, co pozwoliło na stwierdzenie interferencji w oznaczeniu androstendionu. W obu przypadkach postawiono ostateczne rozpoznanie zespołu policystycznych jajników.

Wnioski: W diagnostyce różnicowej zespołu PCO w przypadku zaskakującego wysokiego stężenia androgenów, przy braku nasilonych objawów klinicznych, należy uwzględnić możliwość wystąpienia interferencji w oznaczaniu androgenów.

Key words: hiperandrogenizacja / androjeny / androstendion / /interferencje w oznaczaniu hormonów /
Siemens, USA), with intra-assay CV (range %): 6.2%-15.1%,
inter assay CV: 8.5%-17.8%, analytical sensitivity: 0.3 ng/ml and
assay range: 0.3- 10ng/ml. Serum concentrations of FSH, LH,
edradiol, prolactin, testosterone, DHEAS, cortisol, TSH, free
T4 and free T3 were measured by electrochemiluminescence
immunoassay (ECLIA) method using Elecsys - Cobas e 601
immunoassay analyzer, Roche Diagnostics GmbH Mannheim
Germany. Concentrations of 17-OH progesterone were assessed
by enzyme linked immunosorbent assay (ELISA) method based
on the principle of competitive binding, using commercially
available 17-OH Progesterone ELISA kits (IBL International).

In order to rule out possible androgen-secreting tumour,
we performed a 48-hour dexamethasone suppression test (0.5
mg every 6-hours). This failed to demonstrate suppression of
androstandione (6.05 ng/ml after one day and 9.32 ng/ml after
two days) (see Table I), where criteria for adequate suppression
of androgens during 48hr dexamethasone suppression test are
described by Kaltas et al. 2002 [6]. Pelvic ultrasound
examination showed polycystic ovaries, while abdominal CT
scan failed to show any ovarian or adrenal lesion. A possibility
of androstandione assay interference was therefore raised. Given
the lack of clinical features consistent with such high androstandione
concentrations, we assessed urinary steroid profile by the means
of gas chromatography/mass spectrometry method. This yielded
normal results.

We concluded that such high androstandione concentrations,
out of proportion to clinical presentation, must have resulted
from assay interference. Unfortunately, a direct test for possible
heterophilic antibodies was not possible in our Department. A
final diagnosis of PCOS was established.

Case 2

A 21 years old woman (BMI: 20.1 kg/m²) was admitted for
investigations of oligomenorrhea, persisting since menarche
(at the age of 14). Clinical examination was unremarkable,
and in particular there was no evident hirsutism according to
Ferriman-Gallwey score. Initial hormonal tests revealed: FSH
5.62 IU/l (rr: 2.8-11.3 IU/ml), LH 27.67 IU/l (rr: 1.1-11.6 IU/ml),
eodradiol 127.8 pg/ml (rr: 0-160.0 pg/ml). The above described
hormonal profile corresponded with luteal phase of menstrual
cycle. Concentration of 17-OH-progesterone was also consistent
with luteal phase (2.38 ng/ml (rr: 0.2-2.9 ng/ml)). Further
investigations revealed normal concentrations of prolactin [13.01
ng/ml (rr: 3.9-25.4 ng/ml)], DHEAS 239.1 µg/dl (rr: 148-407 µg/
dl), early morning cortisol 25.44 µg/dl, TSH (0.6 mIU/l), as well
as free T4 and free T3.

Serum testosterone and androstandione concentrations were,
however, significantly above the reference range, i.e. testosterone
0.78 ng/ml (rr: 0.084-0.481 ng/ml) and androstandione above 10.0
ng/ml (rr: 0.3-3.3 ng/ml). Both testosterone and androstandione
concentrations were therefore strikingly high and out of
proportion to patient’s clinical presentation. In order to rule
out possible androgen-secreting tumour or hypercortisolaemia
we performed 48-hour dexamethasone suppression test (0.5 mg
every 6-hours). This failed to demonstrate any suppression of
androstandione (concentrations above 10 ng/ml either after one
and two days) (see Table II). The pelvic ultrasound examination
showed polycystic ovaries, while abdominal ultrasounds
examination failed to demonstrate any adrenal lesion. A possibility
of androstandione assay interference was raised. Given the lack
of clinical features consistent with such high androstandione
concentrations we assessed the concentration of androstandione
after 1:4 and 1:16 dilutions (Table II). Recalculated concentrations
of androstandione were still raised but significantly lower than
predilution concentrations (Table II). Thus, we concluded that
such high androstandione concentrations (i.e. above the upper
limit of the assay) must have resulted assay interference. A final
diagnosis of PCOS was established.

Discussion

Polycystic ovary syndrome is the most common
diagnosable condition in women in reproductive age and is the principal
cause of hyperandrogenism. According to the Rotterdam criteria,
one can recognize PCOS when a patient fulfils two out of three
criteria, i.e. oligo- and/or anovulation, clinical and/or biochemical
signs of hyperandrogenism and polycystic ovaries, however,
only on condition that other causes of oligo-amenorrhea and
hyperandrogenism have been excluded [1,2,3,7,8]. These include
disorders of androgen excess, such as like non-classical congenital
adrenal hyperplasia, Cushing’s syndrome, hyperprolactinaemia,
acromegaly, syndromes of severe insulin resistance, side effects
certain medications [2, 3]. The last but not the least, androgen-
secreting neoplasms must be also excluded [2, 3, 9]. The principal
hormonal evaluation in our patients included serum androgens
(testosterone, androstandione, dehydroepiandrosterone sulfate),
prolactin, thyroid function tests and 17-hydroxyprogesterone
indices. In both patients we observed very high concentrations of
androstandione (i.e. above the upper limit of the assay) as well as
raised concentrations of testosterone, that prompted us to perform
investigations in order to rule out androgen-secreting tumour.

Our first patient had history of oligomenorrhea but did not
have any significant hirsutism, acne or clitoromegaly, defined as
a clitoral diameter of greater than 4 mm [10]. Very high androgen
concentrations, and androstandione in particular, prompted us
to undertake tests in order to rule out an androgen-secreting
tumours. Though these are rare, for instance constitute only 0.2%
cases of hyperandrogenism according to Azizia et al., they are
of paramount clinical importance, while a diagnosis of PCOS
cannot be established, if there is a possibility of an androgen-
secreting tumour [11]. In order to rule out possible androgen-
secreting tumour or hypercortisolaemia we performed 48-hour
low dose dexamethasone suppression test (LDDST), where first
blood samples are collected in the morning , then dexamethasone
is administered at a dose of 0.5 mg every six hours for 48 hours
according to the standard protocol [12]. According to Kaltas et al.,
this test could be also applied to investigation of possible
androgen-secreting tumours [6]. Adequate androgen suppression
(i.e. above 50% of initial values or within the reference range)
in response to glucocorticoid administration effectively excludes
androstandione secreting adrenal of ovarian tumour. In particular,
all patients who harboured an androgen-secreting tumour failed
to achieve normalization of elevated androgen levels during
LDDST or had a greater than 40% reduction from baseline [6].
With respect to the above criteria, the LDDST was associated
with 100% sensitivity and 88% specificity in identifying patients
with androgen-secreting tumours [6].

In our first patient we observed satisfactory suppression of
cortisol [i.e. below 1.8 µg/dl (50 nmol/l)] and DHEAS but...
we did not observe adequate suppression of testosterone and androstendione (0.68 ng/ml compared to 0.68 ng/ml after LDDST) and androstendione (12.7 ng/ml compared to 9.32 ng/ml after LDDST). The results of the test, therefore, allowed us to rule out Cushing’s syndrome, but suggested a possibility of an androgen secreting tumour. Pelvic ultrasonography and abdominal computed tomography imaging were - therefore - performed. The ultrasound scan showed polycystic ovaries, while abdominal CT scan failed to show any ovarian or adrenal lesion. Given the patient clinical presentation, and in particular an absence of obvious signs of hyperandrogenism or virilisation, a possibility of androstendione and testosterone assay interference was raised. Thus, we assessed a urinary steroid profile by the means of gas chromatography/mass spectrometry method. Gas chromatography–mass spectrometry (GC-MS) enables to precisely identify various substances within a test sample, produced during a ionisation process, according to their mass-to-charge-ratio. Mass spectrometry-based methods are currently the most specific quantitative analytical methods for steroids determination [13]. In contrast to immunoassays, this method is independent of matrix effects or cross-reactivity [13]. Taking all this into account, an analysis of urinary steroid metabolite excretion was performed according to the method described by Arlt et al. and Ambroziaik et al., i.e. by a quantitative gas chromatography/mass spectrometry (GC-MS) selected ion-monitoring method [14, 15]. Steroids quantified according to this method include corticosterone metabolites [tetrahydrocorticosterone (THB), 5αTHB, tetrahydro-11-dehydrocorticosterone (THA), and 5αTHA], the progesterone metabolite pregnanediol (PD), 17OHP metabolites [pregnanetriol (PT), and 17-hydroxypregnanolone (17HP)], the 17-hydroxypregnanolone metabolite pregnenetriol (5-PT), the 21-desoxycortisol metabolite pregnantriolone (PTONE), cortisol metabolites [tetrahydrocortisol (THF), 5αTHF, tetrahydrocortisone (THE)], and androgen metabolites [androstenedione, etiocholanolone, dehydroepiandrosterone (DHEA), and 16-hydroxy-DHEA]. Despite very high androstendione concentrations (i.e. above the upper limit of the immunoassay), all steroids metabolites were within normal range when measured by GC-MS method, while in case of such very high androstendione concentrations one might expect an increase in androstendione metabolites, i.e. androsterone, epitostosterone and etiocholanolone. Hence, we concluded that such high androstendione concentrations must have been caused by assay interference.

In the second presented case, again there was an absence of significant clinical features or hyperandrogenism or virilisation, contrasting with high testosterone, and particularly very high androstendione concentrations. There was also an inadequate suppression of testosterone and androstendione concentrations during LDDST. In this case, however, we performed serial dilutions of androstendione sample, where recalculated androstendione concentrations were significantly lower, i.e. there was very poor recovery of initial androstendione concentrations. Such result, in our opinion, confirmed an interference in androgen assay.

There are several possible causes of interference with androgen assays. For instance, the presence of heterophilic antibodies can cause interference with several immunoassays,
including androgen assays [16]. Unfortunately, in our Department, we had no access to blocking antibodies in order to confirm or refute this hypothesis (i.e. to measure androstenedione and testosterone concentrations before and after addition of blocking antibodies). Thus, though a possibility of heterophilic antibody interference is highly likely, it still remains speculative. It is also recognised that cross-reactivity with other metabolites can disturb the androgen evaluation. The most common metabolite which might cause interference is spironolactone. Namely, treatment with 100-200 mg/d of spironolactone was associated with spuriously high androstenedione concentrations measured by an immunoassay [17]. Also norethisterone has recently been reported to cause interference in androgen assays [18]. However, our patients were not receiving spironolactone or norethisterone, though obviously we cannot fully exclude a possibility of interference by other steroid metabolites. It should be also noted that our second patient (aged 21) had already passed her adolescent age, so standard criteria for diagnosis of PCOS could be applied [19].

Conclusions

In the absence of clinical features, contrasting with unusually high androgen levels, a possibility of androgen assay interference should be considered in differential diagnosis of hyperandrogenism and/or PCOS. Such interference may prompt costly, and eventually unnecessary, investigations in order to rule out androgen-secreting tumour. It is also a reminder that grossly abnormal results of laboratory investigations must not be interpreted outside a clinical context.

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