CASE REPORT

Negative urine pregnancy test in a molar pregnancy: is it possible?

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SUMMARY

A urine pregnancy test is commonly used to detect pregnancy and is based on finding intact β -human chorionic gonadotrophin (hCG) molecules in the urine by an immunoassay system. However, the significantly large amount of β -hCG in molar pregnancy may paradoxically lead to a false-negative result due to a phenomenon known as the 'high dose hook effect'. A case of molar pregnancy with negative urine pregnancy test but very high serum β -hCG is reported. Every obstetrician should be aware of this limitation in the presence of a high index of suspicion of gestational trophoblastic disease but negative urine pregnancy test.

BACKGROUND

Gestational trophoblastic disease (GTD) is a spectrum of pregnancy-related disorders arising from abnormal placental trophoblastic proliferation with the potential of invasion and metastasis. All forms of GTD produce human chorionic gonadotrophin (hCG), the amount of which correlates with the disease volume. hCG is an important biomarker for diagnosis and follow-up of women with GTD. The diagnosis of GTD is usually performed by positive urine pregnancy test with snow storm pattern in ultrasound. However, the significantly large amount of hCG in molar pregnancy may paradoxically lead to a false-negative result in some urinary hCG immunoassay systems commonly used for pregnancy screening due to a phenomenon known as the 'high dose hook effect'.

CASE PRESENTATION

A 24-year-old woman, para one with one live birth, presented with symptoms of mild vaginal bleeding for 2 days following 2 months of amenorrhoea. Her previous menstrual cycles were regular. She was using barrier contraception since her last delivery, which was a full-term normal vaginal delivery 2 years earlier with no antenatal or postnatal complications. There was no history of headache, disturbed vision or galactorrhoea.

The patient was haemodynamically stable with pulse rate of 88 bpm and blood pressure of 118/70 mm Hg. There was no pallor or lymphadenopathy. Abdominal examination revealed a suprapubic lump corresponding to an 18-week gravid uterus. It was central, non-tender, with a smooth surface and a regular outline. Speculum examination revealed a healthy cervix and vagina with slight blood-stained vaginal discharge. Vaginal examination confirmed it to be an 18-week-old sized uterus.

INVESTIGATIONS

The patient's bedside urine pregnancy test was performed with an over the counter device (Rapid pregnancy test card from Apollo pharmacy), and was negative. Ultrasound ([figure 1](#)) revealed an enlarged uterus measuring 22 cm $\times$ 10 cm $\times$ 9 cm with a heterogeneous vascular mass of 18 cm $\times$ 8 cm $\times$ 8 cm occupying the whole uterine cavity with multiple cystic areas within the mass giving a 'snowstorm appearance' suggestive of a molar pregnancy. Bilateral ovaries were enlarged with multiple cysts. The bedside urine pregnancy test was repeated and was again negative. In view of the ultrasound finding, quantitative serum β -hCG estimation was performed by immunochemiluminometric assay in the laboratory and came to 1 154 830 mIU/mL. The patient's haemoglobin was 10.5 gm/dL with a normal platelet count. The coagulation profile, liver and kidney function test, serum thyroid-stimulating hormone and chest X-ray were within normal limits.

DIFFERENTIAL DIAGNOSIS

Molar pregnancy, degenerated fibroid if serum β -hCG is negative.

TREATMENT

Suction evacuation was done. A large amount of grape-like vesicular tissue was evacuated. The histopathological examination confirmed a complete hydatidiform mole.

OUTCOME AND FOLLOW-UP

The patient's serum β -hCG was followed up weekly. It turned negative in 8 weeks.

Figure 1 Ultrasound showing multiple cystic areas in the uterine cavity giving a 'snowstorm appearance' suggestive of molar pregnancy.

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DISCUSSION

Hydatidiform mole is the commonest form of GTD with a very high incidence in Asian countries.¹ It generally affects women of maternal age ≤ 15 or ≥ 40 years.

Clinical diagnosis of any woman of childbearing age presenting with amenorrhoea, abdominal pain or vaginal bleeding requires exclusion of pregnancy, which is performed by the commonly available over-the-counter urine pregnancy test kits based on detection of hCG in urine. hCG is a glycoprotein hormone with two non-covalently linked α and β subunits. The immunoreactivity of hCG in urine during pregnancy is because of intact hCG molecules (intact heterodimer comprising α and β subunits) as well as partially degraded variants detectable in serum and urine. Most over-the-counter urine pregnancy test kits detect intact hCG molecules only at concentrations of ≥ 25 mIU/mL². These kits are chromatographic sandwich immunoassays in which the hCG molecules present in the urine sample react with migratory colloidal gold particles coated with an anti- β -hCG antibody, the product then migrates by capillary action to a fixed detection line coated with an anti- α -hCG antibody to induce colour change.

False-negative urine pregnancy tests have been reported previously. The most intuitive reason for this false-negative test is dilute urine.³ A false-negative urine test can also occur with 'high dose hook effect', which probably occurred in this case. It occurs when very high concentrations of hCG, present in urine, saturate the solid migratory phase as well as the fixed detection antibodies, independently.⁴ Hence, excessive levels of free antigen in the sample allow the anti- β -hCG and anti- α -hCG antibodies to bind subunits of different hCG molecules rather than subunits of the same molecule, preventing them from forming the 'sandwich.' As a result, the gold particle necessary for colour change is not bound, resulting in a false-negative test. This hook effect can be overcome by diluting the sample, thereby reducing the concentration and allowing the antibodies to properly bind to two portions of the same molecule.⁵ The hook effect is not specific to urine and has been documented in the serum also, especially when the serum β -hCG concentration is more than 1 000 000 mIU/mL.⁶

The false-negative test may also occur due to a 'variant hook effect', which is more common than the hook effect.⁷ As pregnancy advances, in addition to intact hCG molecules, variant forms of hCG also begin to appear in the serum as well as urine. These variants are core fragments of β -hCG (hCG β cf), hyperglycosylated hCG, hCG missing the β subunit C-terminal

peptide, free β subunit and nicked hCG. The amount of these variants present in urine changes during different trimesters of pregnancy. The hCG-h is high in early pregnancy and hCG β cf is high in midterm pregnancy in urine.⁸ The high concentration of hCG β cf can saturate one of the antibodies used in pregnancy test kits and no sandwich forms as the other antibody does not recognise the β core fragment and the pregnancy test appears negative.

Learning points

- ▶ A urine pregnancy test can be falsely negative in molar pregnancy because of the high dose hook effect or variant hook effect.
- ▶ Definitive diagnosis requires quantitative serum β -human chorionic gonadotrophin estimation, which may also be falsely negative because of the hook effect.
- ▶ Dilution of urine or serum or both should be done in such cases to overcome the hook effect.

Competing interests None.

Patient consent Obtained.

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