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Case Report

False-negative qualitative human chorionic gonadotropin (hCG) test result ('hook effect') with classical ultrasound findings of complete molar pregnancy: an uncommon case

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Abstract

False-negative qualitative Human Chorionic Gonadotropin (hCG) result is a phenomenon in which large amounts of β-hCG are produced by molar pregnancy, oversaturating the test's assay system and leading to false-negative results known as the 'prozone phenomenon' or the 'hook effect'. This can lead to misdiagnosis and delay in management despite high suspicious clinical and ultrasound findings. We report a case of an 18-year-old female who presented to our health facility with amenorrhea of 16 weeks, lower abdominal pain, soft and large fundal height for gestational age, and cramping with slight per-vaginal bleeding, and a negative urinary pregnancy test (UPT). Based on clinical presentation, ultrasound findings and a positive UPT after urine dilution, molar pregnancy was diagnosed. Aspiration was performed under ultrasound guidance, and follow-up was done as per MSF guidelines. HCPs need to be familiar with some rare cases for which the possibility of finding false-negative UPT is likely.

INTRODUCTION

Gestational trophoblastic disease (GTD) encompasses a spectrum of tumours, including molar pregnancy and other trophoblastic neoplasms. The non-molar or malignant forms of GTD are called gestational trophoblastic neoplasia (GTN), which includes various histological tumour types (invasive mole, choriocarcinoma, placental site trophoblastic tumour, and epithelioid trophoblastic tumour). Molar pregnancy produces characteristic clinical features, including vaginal bleeding and uterine size beyond expected gestational age [1–3]. Other clinical features of molar pregnancy, including hyperemesis gravidarum and hyperthyroidism, are believed to be induced by markedly elevated serum levels of human chorionic gonadotropin (β-hCG) produced by the trophoblastic tissue. Thus, a hallmark of diagnosing molar pregnancy is a positive β-hCG-assay urinary pregnancy test (UPT), in addition to a complete physical and pelvic examination, complete blood count, blood chemistry, and pelvic ultrasound. Interestingly, UPTs can produce false-negative results in the presence of high antigen concentrations (known as 'hook effect') [4]. We report a case of molar pregnancy presenting as abdominal pain and vaginal spotting with multiple false-negative UPTs.

CASE REPORT

An 18-year-old, nulliparous woman from the Kigoma region, Tanzania, and married to a man aged 24 years, presented to a primary health-care facility as primigravida in March 2021. She had had sexual intercourse with her husband without contraception. Her menstrual cycle had been regular, until her last normal menstrual period on Jan 27, 2021. At her first antenatal clinic visit (March 1), she had a positive UPT, other basic urinary dipstick tests were negative as was malaria rapid diagnostic test (RDT), haemoglobin level was 13.3 g/dl, blood pressure (BP) was 110/63 mmHg, and the uterus was palpable above the symphysis pubis.

At the second visit (May 25), the patient reported not feeling foetal movements. Fundal height was 20 cm (versus expected 14–18 cm). Urine dipstick, malaria, and syphilis tests were negative. A UPT was negative. With no signs of vaginal bleeding, leakage, or a serious illness in previous months, the patient was advised to go home.

1 week later (June 1), she presented to the same facility with lower abdominal pain and cramps, slight per-vaginal bleeding, and feeling of abdominal fullness. She was referred to the MSF health centre with suspected intrauterine fibroids.

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Figure 1. Transabdominal ultrasound scan before manual vacuum aspiration with snowstorm appearance.

Figure 2. Urinary pregnancy test results after serial dilution of urine.

At the MSF facility, her BP was 148/91 mmHg, temperature 37.3°C, heart rate 117 bpm, respiratory rate (RR) 16 breaths/minute, and bodyweight 45 kg, haemoglobin level 8.0 g/dl, and blood glucose (BG) 3.4 mmol/dl.

She had a slight bilateral lower limb oedema with some paleness of conjunctiva and palms. Abdomen examination revealed a uterus palpable above the umbilicus, soft and large, and with a fundal height of 24 cm. Foetal Doppler machine revealed no foetal cardiac activity. Per-vaginal examination revealed normal cervix and vaginal mucosa and some minimal bleeding from the cervix.

DIAGNOSIS

A point-of-care obstetric ultrasound revealed an intrauterine heterogeneous vesicular connected with a cluster of grapes' aspect, filling the entire uterus (Fig. 1). The pouch of Douglas had no free fluids, and the ovarian follicles were enlarged on both sides. These findings were strongly suggestive of a complete molar pregnancy.

On June 1, after another negative UPT, the test was repeated with a serial dilution of the urine (40 ml urine+10 ml water [dilution factor, DF, 1.25]; followed by 40 ml first dilution+10 ml water [DF 1.5], and so on). Diluted samples (Fig. 2) showed negative results until the fifth dilution (DF 2.25), which was positive.

The multidisciplinary team recommended the use of a manual vacuum aspiration technique to remove the molar tissue, for which the patient consented.

A blood transfusion (2 units) was given before the procedure due to anaemia. Haemoglobin level was controlled after 12 h (13.1 g/dl). The patient was orally given misoprostol for cervical ripening (400 μg, 3 h before the procedure), doxycycline (200 mg, 1 h) and ibuprofen (400 mg, 1 h).

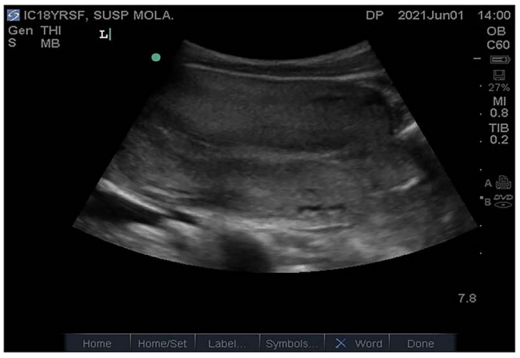

Figure 3. Transabdominal ultrasound scan after manual vacuum aspiration.

During the procedure, the patient received oxytocin (20 IU) in ringer lactate (1 l) intravenously (at 160 drops/min) to reduce the risk of uterine perforation and bleeding [5]. The aspiration of uterine contents was done under ultrasound guidance. Approximately 2 l of aspirants were evacuated (Fig. 3).

The patient was put under 24 h observation. The last evaluation showed a BP of 115/69 mmHg, temperature 36.7°C, heart rate 73 bpm, RR 15 breaths/minute, haemoglobin level 11.2 g/dl, and BG 3.3 mmol/dl.

On discharge, the patient was counselled on contraceptive methods and opted for a 3-year implant method. An ultrasound showed no retained tissue in the uterus 2 weeks post procedure. As per MSF protocol [5], the patient took a series of UPTs every 4 weeks, and the results were negative for 12 consecutive tests, suggesting complete molar evacuation.

DISCUSSION

Symptoms of gestational trophoblastic disease include nausea and vomiting, abnormal vaginal bleeding, or an enlarged uterus. In this case, an ultrasound was done because of the enlarged uterus and the vaginal bleeding, but the diagnosis may be missed in cases without these two signs and with a negative UPT. Thus, recording of detailed history and clinical examination should be considered as a gold standard when managing similar cases.

In low-resource settings where quantitative β-hCG assays and histology are not easily accessible, ultrasound and clinical signs could help guide diagnosis of complete molar pregnancy. Molar pregnancy can be highly suspected if transabdominal ultrasound shows a snowstorm sign, an endometrial mass with multiple anechoic spaces, no foetus, and no amniotic fluid. This abnormal scan is unlikely to be missed [4, 6], but these features should not be relied on for diagnosis when evaluating molar pregnancies in first-trimester pregnancies [7].

The current urine and serum pregnancy tests are known as 'Sandwich' assays. When present, hCG is immobilized by a capture antibody and labelled by a tracer antibody, resulting in an antibody-hCG-tracer 'sandwich'. When hCG levels are high (>500 000 mIU/ml) [8], both the capture and tracer antibodies saturate, and non-sandwiched tracer antibodies are washed away with the excess material, resulting in a falsely low or negative test ('hook effect') [9].

Although the 'hook effect' is rare in molar pregnancy, if suspicion is high, clinicians need to carry out sample dilutions (DF $\geq$ 2.25 dilution) to obtain a positive test. Similar observations have been reported previously, where dilution

of the serum or urine sample was done to overcome that phenomenon [8, 10]. Prompt evacuation of uterine contents is the mainstay of treatment, and follow-up can be done with UPT, which in this case was cost-effective and accessible for the patient and led to successful monitoring after molar pregnancy aspiration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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ETHICS APPROVAL

Ethics approval was given by the Regional Medical Office in KIGOMA region, Tanzania.

CONSENT

It was written and informed.

GUARANTOR

Daud Phillipo and Jean Kalibushi Bizimana.

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