Case Report

"High-dose hook effect" - negative urine β-HCG in molar pregnancy

Jain R, Agrawal S, Baid M[1], Jain A[2], Baid M[3]

Departments of Obstetrics and Gynaecology and [1]Urology, S.P. Medical College, Bikaner, [2]Department of Obstetrics and Gynaecology, Mahatma Gandhi Medical College, Jaipur, Rajasthan, [3]Department of Orthopedics, Medica Superspecialiy Hospital, Kolkata, West Bengal, India

ABSTRACT

The hydatidiform mole, commonly known as molar pregnancy is characterized by the abnormal proliferation of trophoblasts. A hallmark of diagnosing hydatidiform mole is a positive β-hCG assay pregnancy test. Currently used β-hCG assays are sandwich chromatographic immunoassays and may sometimes produce false-negative results in the presence of excessively high antigen concentrations, a phenomenon known as the "high-dose hook effect". We report an atypical case of molar pregnancy in a 24-year-old primi gravida female who presented to us in emergency department with a huge abdominopelvic mass, respiratory distress and bilateral pedal edema with a negative urine pregnancy test. Ultrasongraphy suggested it to be hydatiform mole and on further evaluation serum β-hCG was found to be raised. Repeat urine β-hCGassay showed positive result on 1:10 dilution. After resuscitation, patient was treated with suction and evacuation which demonstrated grape like vesicles with no fetal parts. Histopathology report revealed it to be complete hydatiform mole.

Key words: Hook effect; β-hCG assay; molar pregnancy.

Background

The hydatidiform mole, commonly known as molar pregnancy is characterized by the abnormal proliferation of trophoblasts, the cells that normally develop into the placenta.[1] These degenerated trophoblasts have a vesicular appearance and have been described to resemble a "bunch of grapes."[2] A hallmark of diagnosing hydatidiform mole is a positive β-hCG assay pregnancy test. Currently used β-hCG assays are sandwich chromatographic immunoassays and may sometimes produce false-negative results in the presence of excessively high antigen concentrations, a phenomenon known as the "high-dose hook effect." We report a case of molar pregnancy who presented in labor emergency room with respiratory distress, a huge abdominopelvic mass, and bilateral pedal edema with a negative urine pregnancy test.

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Case Description

A previously healthy 24-year-old primi gravida female presented to the emergency department (ED) with 3 months amenorrhea with mass per abdomen with breathlessness, pain lower abdomen and bilateral pedal edema for last 4 days. She also gave history of vaginal bleeding 1 month back which lasted for 3 days and stopped spontaneously. Pain was continuous dull aching in nature and was not associated with any aggravating or relieving factor. There was no history of any fever, burning sensation of urine or diarrhea. She tested for pregnancy at 2 months of amenorrhea using a urine pregnancy kit at home which turned out to be negative. She

Address for correspondence: Dr. Jain R,
Department of Obstetrics and Gynaecology, S.P. Medical College, Bikaner - 334 001, Rajasthan, India.
E-mail: drruchijainbaid@gmail.com

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was treated at a local hospital before being referred to our emergency department.

On examination, she was pale with generalized edema, a heart rate of 150 bpm, blood pressure of 180/100 mmHg, respiratory rate 22 per minute, oral temperature of 98.3° F and oxygen saturation of 100%. Bilateral crepitations were noted in lungs. Examination of cardiovascular system and other systemic examination were normal. On palpation she had a large, tense, and diffusely-tenderabdominal mass corresponding to 24-week gestation. On speculum examination mild bleeding was noted. On per vaginal examination cervical os was closed with blood mixed discharge and a 22-week size uterus with fullness felt in all fornices. Urine β-hCG assay was done and it turned out to be negative. Technical error was suspected and test was repeated, still the result was negative. Ultrasound whole abdomen and pelvis, complete hemogram, blood group, renal function test, and liver function test were done. Ultrasonography showed grossly enlarged uterus with heteroechoic area showing multiple variable size cysts and a large cystic area of about 12 cm × 3 cm with no evidence of fetal tissue, suggestive of vesicular mole. Bilateral ovaries were found enlarged with few cystic lesions of 5.5 × 2.3 cm in left ovary and of size 6.9 × 4.2 cm in right ovary suggestive of theca lutein cysts. Compete blood count revealed hemoglobin to be 5 gm/dl, total WBC count was 14000/mm^3 with neutrophil 84.8%, lymphocytes 11.9%, eosinophil 0.4%, monocytes 2.8% and basophils 0.1% and normal platelet count. Her coagulation profile was normal. Fasting blood sugar was 94 mg/dl, blood urea 24 mg/dl, serum creatine 0.95 mg/dl total bilirubin 1 mg/dl, alkaline phosphatase 127U/l, SGOT 27u/l and SGPT 34 u/l. Her blood group was found to be O positive. Quantitative serum β-hCG and thyroid profile were also ordered. Serum showed T3 1.86 ng/ml, T4 10.3 µg/dl and TSH 0.45 mIU/ml. Serum β-hCG was found to be 2,55,000 mIU/ml. After diagnosing molar pregnancy on ultrasound, [Figure 1a,b] chest X-ray was done which showed bilateral minimal pleural effusion. ECG and 2D Echo were done and found to be normal. Pregnancy test was repeated with dilution of the urine sample and positive result was obtained with 1:10 dilution.

She was treated with prophylactic dose of magnesium sulphate and anti hypertensives for severe pre-eclampsia. Three units of blood were transfused. Once stabilized, patient was taken to the operating room for suction and evacuation under general anesthesia, which demonstrated grape-like vesicles with no fetal parts [Figure 2]. Oxytocin drip was started to prevent hemorrhage. Post-operative period was uneventful and was discharged on post-operative day 4. Serum β-hCG trended downwards from 2,25000 mIU/ml on days 1 and 2 postoperatively to 70,500 mIU/ml on day 4. Histopathology report revealed complete hydatidiform mole. Despite of being advised patient never returned for further follow up.

Discussion

Gestational trophoblastic disease encompasses a spectrum of tumors, including complete and partial hydatidiform mole (molar pregnancy) and locally invasive or disseminated choriocarcinoma.[3] In a complete molar pregnancy, there is diffuse trophoblastic proliferation and hydropic degeneration of chorionic villi. There's no fetal tissue formation. In a partial molar pregnancy, there is focal trophoblastic proliferation and presence of fetal tissue or amniotic sac.

Gestational trophoblastic disease demonstrates marked geographic and ethnic differences, with the highest incidence in South-East Asia.[1] It is most commonly associated with pregnancy in the early (15-20 years old) and late (>35 years old) reproductive periods.[4] A retrospective analysis reported that 75% of patients present with vaginal

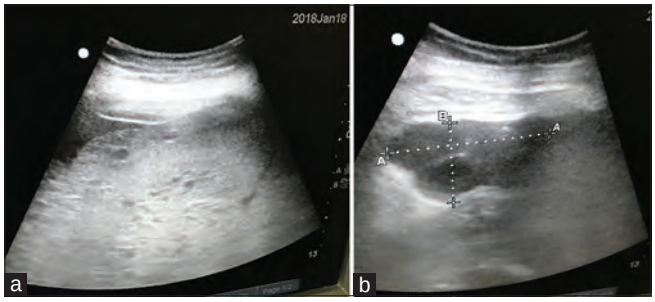

Figure 1: (a) Ultrasonography image showing grossly enlarged uterus with heteroechoic area with multiple variable size cysts with no evidence of fetal tissue, suggestive of complete hydatidiform mole. (b) Ultrasonography image showing enlarged left ovary with cyst of size 5.5 × 2.3 cm suggestive of theca lutein cyst

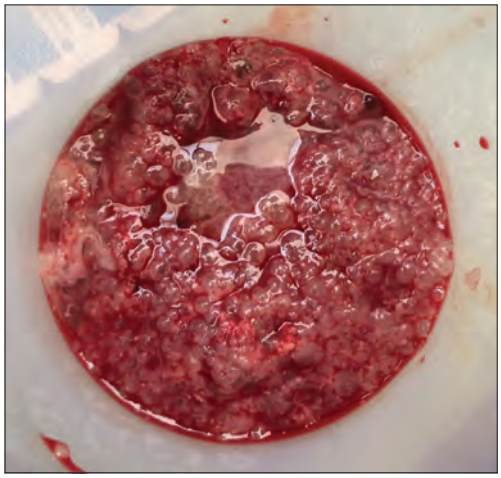

Figure 2: Suction and evacuation specimen showing grape like vesicles with no fetal parts

bleeding, while 54% present with enlarged uterus for gestational dates and 100% had excessively elevated β-hCG levels.[5]

Complete hydatidiform mole produces characteristic clinical features, including vaginal bleeding and uterine size beyond expected gestational age. Many other clinical features of molar pregnancy, including hyperemesis gravidarum, hyperthyroidism, and theca lutein ovarian cysts are believed to be induced by markedly elevated serum human chorionic gonadotropin (β-hCG) levels produced by the trophoblastic tissue. Approximately 50% of patients of complete hydatifrom mole have hCG levels >100,000 mIU/mL.[6] Yeung and Cheung have reported that at serum levels of hCG above 500,000 mIU/mL, a "hook effect" can occur, resulting in an artificially low or negative value when using the current commercially available immunometric hCG assays.[7] However, in our case, this effect was seen at serum β-hCG value of 2,55,000 mIU/ml.

Clinical decision-making regarding women of childbearing age with abdominal pain and vaginal bleeding is often dictated by urine qualitative pregnancy hCG assays. These assays are chromatographic sandwich immunoassays in which two antibodies directed to different portions (for example, the α and β subunits) of the hCG molecule sandwich a single antigen to produce color change. This lateral flow, chromatographic assay is reported to produce positive results with hCG concentrations $\geq$25 mIU/mL. A urine sample placed on the membrane reacts with migratory colloidal gold particles coated with anti-β-hCG antibodies. These antibody-bound particles then migrate by capillary action to the fixed detection line coated with either anti-α-hCG or goat-anti-mouse antibody (control). To induce color change a single hCG molecule must be bound by the antibodies to both subunits, forming a "sandwich" that attaches the gold particles for color change and binds the compound to the detection line.

Despite the high sensitivity and specificity of the assay, our patient had negative urine pregnancy testupon presentation to our ED. One explanation for the negative pregnancy test is the "high-dose hook effect," also known as "prozone phenomenon", a rare phenomenon that occurs in sandwich immunoassays when the concentration of the antigen is sufficiently high to saturate both the solid migratory phase and fixed detection antibodies independently. In this case, excessive levels of free antigen in the sample allow the anti-β-hCG and anti-α-hCG antibodies to each 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 color change is never bound, leading to a false-negative test. A 1:10 to 1:1000 dilution of the antigen sample may overcome the hook effect by reducing the concentration and allowing the antibodies to properly bind to two portions of the same molecule.[8]

A retrospective analysis by Coukos GC *et al.*, has suggested that the positive predictive value of transvaginal ultrasound for molar pregnancy is 100%.[5] However, often this procedure is delayed or not considered when a pregnancy test is negative. This shows the importance of quantitative β-hCG assays and sonographic evaluation in patients with negative pregnancy tests where clinical suspicion of pregnancy remains high.[9]

Conclusion

Although modern assay methods have much improved reliability in the diagnosis of molar pregnancy, physicians should still be aware of the potential for false-negative urinary and serum β-hCG results due to the "high-dose hook effect". So negative or inconclusive results in patients with high clinical suspicion of pregnancy should be further evaluated by serum quantification of β-hCG and sample dilution in order to prevent delayed diagnosis and related complications.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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