CASE REPORT

Limitations of the Human Chorionic Gonadotropin (hCG) Assay in the Diagnosis of Gestational Trophoblastic Disease

Zety Wizana Rusman[1,2], Subashini C. Thambiah[1], Intan Nureslyna Samsudin[1], Siti Sharina Anas[3], Pavai Staneshwar[4]

[1] Department of Pathology, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia
[2] Department of Pathology, Hospital Kuala Lumpur, Malaysia
[3] Department of Pathology, Hospital Putrajaya, Wilayah Persekutuan Putrajaya, Malaysia
[4] Division of Laboratory Medicine, Department of Pathology, University Malaya Medical Centre, Kuala Lumpur, Malaysia.

ABSTRACT

A 31-year-old lady presented with abnormal vaginal bleeding during her first trimester of pregnancy. Based on the ultrasound findings and the decreasing hCG trend (from 1040 mIU/mL to 759 mIU/mL), a diagnosis of missed miscarriage was made. A week later, the patient presented with heavy vaginal bleeding. Ultrasound findings showed classic snowstorm appearance suggestive of complete hydatidiform mole. The serum hCG level however, was 357 mIU/mL. In a case of hydatiform mole with inappropriately low hCG, analytical interference was suspected. Post-dilution serum hCG of 5,775,000 mIU/mL confirmed the presence of hook effect in a two-site hCG immunoassay. Discordance between serum hCG and clinical findings should be actively investigated by the laboratory to prevent delay in diagnosis and treatment. This case also highlights the need for clinicians to be aware of the hCG assay used in their hospital's laboratory so that they may recognise false negative hCG results.
Malaysian Journal of Medicine and Health Sciences (2022) 18(SUPP21): 134-136. doi:10.47836/mjmhs18.s21.22

Keywords: Hydatidiform mole, Gestational trophoblastic disease, Human chorionic gonadotropin, Two-site immunoassay, Hook effect

Corresponding Author:
Subashini C. Thambiah, MPath
Email: subashini@upm.edu.my
Tel: +60123923709

INTRODUCTION

Gestational trophoblastic disease (GTD) is a disorder of placental trophoblastic epithelium proliferation. In molar pregnancy or hydatidiform mole, a premalignant spectrum of GTD, the serum hCG levels are markedly high compared to the gestation period (1). Identification of different molecular forms by different assays and assay interferences are recognised issues in hCG measurement (2).

CASE REPORT

A 31-year-old gravida 5, para 3+1 in her 11th week of pregnancy, presented with a two-day history of vaginal bleeding. The patient had a history of complete miscarriage three months prior to this pregnancy. Physical examination revealed a soft abdomen with an enlarged uterus of 14 weeks gestational size. A vaginal examination revealed a healthy cervix with closed os and absence of product of conception (POC) in the cervical canal. Her vital signs were stable and examination of all other systems were unremarkable.

The transabdominal (TAS) and transvaginal (TVS) ultrasound scans showed presence of an intrauterine gestational sac (IUGS). Serum hCG level was 1040 mIU/mL and not corresponding to the gestational age. The TAS repeated the next day by a different clinician revealed an IUGS with crown rump length of six weeks gestational age but undetectable foetal heart activity. The serum hCG level four days later was 759 mIU/mL. Based on the ultrasound findings and the decreasing trend of serum hCG, a diagnosis of missed miscarriage was made. The next appointment was in a fortnight to review her symptoms and repeat TAS.

However, one week later, the patient presented with heavy vaginal bleeding. She was pale and her haemoglobin concentration was 8.5 g/dL. TAS and TVS showed classic snowstorm appearance suggestive of complete hydatidiform mole and presence of bilateral multiloculated ovaries with no free fluid. The serum hCG level was 357 mIU/mL. This diagnosis was communicated to the laboratory by the clinician. In a case of hydatiform mole with inappropriately low hCG level, analytical interference was suspected. Due to this discrepancy, the laboratory performed a serum dilution of 1:500 resulting in hCG of 5,775,000 mIU/mL. The

previous two samples were retrieved and hCG was measured after 1:200 dilution. The post diluted results for both samples were >1350 mIU/mL. Further dilution could not be done due to insufficient volume (Table I).

The patient underwent suction and curettage (S&C). Post S&C, serum hCG level decreased to 314,500 mIU/mL post-dilution and serial monitoring revealed decreasing trend although persistently high. Due to persistently elevated hCG levels, a second S&C procedure was performed. Two weeks later, serum hCG was still noted to be raised at 24,713 mIU/mL (Table II). Hence, the patient was diagnosed as having persistent GTD and was referred to a tertiary oncology centre for further management.

DISCUSSION

Trophoblastic tumours are highly heterogenous and although most of them produce intact hCG, various other hCG variants (particularly β-hCG) are also produced (2). This case illustrates the hook effect phenomenon, which is a recognised interference of two-site immunoassays (3). Falsely low serum hCG was suspected in this patient as the level did not correlate with the clinical and ultrasound findings.

First trimester vaginal bleeding is the most common presentation of hydatidiform mole. Ultrasonography and serum hCG levels are useful in distinguishing different causes of early pregnancy haemorrhage, which include miscarriages, ectopic and molar pregnancy (1). In this patient, an initial diagnosis of missed miscarriage was made due to the combination of ultrasound findings with inappropriately low serum hCG for the gestational period, and failure of doubling in serum hCG concentration. The subsequent discordance between serum hCG and the ultrasound findings after a week led to the suspicion of hook effect (3). On serial hCG monitoring, this patient was diagnosed with persistent disease as her serum hCG remained >20,000 mIU/mL. Persistent GTD is defined as serum hCG that plateau or rise during the three consecutive weeks following evacuation of molar tissue or levels that are persistently >20,000 mIU/ml (1).

Quantitative measurement of serum hCG is not harmonised due to hCG's heterogeneity. Different immunoassay platforms can yield different hCG results when the same sample is tested. This may be due to the presence of different hCG variants in serum, employment of different epitope specificities in different assays or variation in the purity of secondary standards used for hCG calibration by different manufacturers. Due to the above factors, monitoring of a patient is best done using the same hCG assay (2).

The assay used to measure serum hCG in this patient was the Beckman Coulter Access Total β-hCG (5th IS [International Standard]), a sequential two step immunoenzymatic 'sandwich' assay. The fundamental difference between this assay and its previous generations is the primary calibrator material. Due to its high level of purification, this calibrator contains less cross-reacting peptides and non-intact hCG variants than earlier IS generations (4).

This assay, however, has its limitations. The manufacturer's product information claimed that the 5th IS assay is only intended for use as an aid in the early detection of pregnancy (5). However, individuals at risk for persistent POC, GTD, and abnormal pregnancies are also serially monitored using hCG testing (4). Two-site immunoassay is prone to high dose hook effect (3). This assay, according to the manufacturer, has no detectable hook effect up to 1,000,000 mIU/mL (5). In this case, the hook effect occurred due to the simultaneous saturation of both labelled and capture antibodies by the markedly high hCG of 5,775,000 mIU/mL. This resulted in incomplete formation of 'sandwich' complexes hence decreased the signal created leading to falsely low result. Furthermore, the washing step in this assay is only included after the sandwich is formed between the analyte, capture antibody and conjugate antibody (5). To reduce the likelihood of hook effect, the washing step should be included before the second antibody is added to the reaction vessel, which will then remove the excess analyte (3). However, this would require modification of the assay. Alternatively, to reduce the risk of releasing falsely low results where there is no warning flag, Al-Mahdili et al suggested to restrict the

Table I: Serial serum hCG pre- and post-dilution

Serum	4/10/18		8/10/18		15/10/18		16/10/18 (Post 1st S&C)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
hCG (0-5) mIU/mL	1040	>1350	759	>1350	357	5,775,000	622	314,500

Table II: Serial serum hCG for monitoring of hydatiform mole post S&C

Serum	22/10/18	30/10/18	12/11/18	16/11/18 (Post 2nd S&C)
hCG (0-5) mIU/mL	169,700	139,300	20,697	24,713

assay's reporting range so that higher concentrations are needed to get the signal down to the reportable range (3). Another cause for falsely low results is the production of other hCG variants not detected by the assay. This 5th IS assay, however, recognises other hCG variants such as intact hCG, the β-hCG, nicked intact hCG and nicked β-hCG variants (5).

The narrow analytical measuring range (AMR) of this 5th IS assay (0.6 to 1350 mIU/mL), when compared to other commercial hCG assays, is one of its major shortcomings (4). Automated assay dilution will only be performed if the measurement exceeds the upper limit of the AMR. The onboard dilution feature allows samples to be quantitated up to approximately 270,000 mIU/mL (5). This explains why manual dilution was required for the patient's sample which was >5,000,000 mIU/mL. The majority of hCG-positive specimens will need to be diluted, and results of manual and onboard dilutions may differ. To check consistency at higher concentrations, laboratories are advised to use quality control material that needs onboard dilution. An ideal hCG assay should have a wide AMR so that true results can be obtained without the need to repeat analysis after dilution (4).

This 5th IS assay has been reported to have a positive bias hence patients undergoing serial monitoring from another platform require re-baselining or alerting the clinician that there will be an increase in quantitative values compared to the previous assay results (4). Other limitations include immunoassay interferences by heterophile antibodies or anti-animal antibodies present in the patient's sample (5). Therefore, clinicians should be aware of these limitations if the hCG assay is utilised for non-pregnancy testing (4). More robust analytical evidence should support the selection of hCG assays for oncology applications.

The laboratory should be informed if a diagnosis of GTD coupled with increased hCG levels is suspected when low hCG values have been reported so that analysis can be carried out on a diluted serum sample. This case demonstrates the significance of clinicians being knowledgeable about the hCG assay utilised in the hospital laboratory and what variants are actually measured so that they may recognise false negative hCG results when they occur in the presence of markedly raised hCG levels as in this patient with hCG level >5,000,000 mLU/mL. To bridge this gap in communication, Catharine Sturgeon in Gronowski (2009) recommends to include in brackets following the method in hCG reporting of results whether the assay is limited to pregnancy use (measures intact hCG only) or it is also appropriate for oncology applications as it has broad specificity (2).

CONCLUSION

To avoid misdiagnosis and consequent poor management of patients, it is crucial to exercise clinical judgement based on the patient's clinical and radiological findings and to be more aware of the analytical limitations of hCG immunoassays. Since it's crucial for clinicians to understand what the hCG assay in their practice actually measures, it is the obligation of the laboratories to make sure the test they offer is fit for the intended clinical use.

ACKNOWLEDGEMENT

The authors would like to thank the Director General of Health Malaysia for his permission to publish this article.

REFERENCES

1. Loh KY, Sivalingam N, Suryani, MY. Gestational Trophoblastic Disease. Med J Malaysia. 2004; 59(5): 697-703.
2. Gronowski AM. Clinical Assays for Human Chorionic Gonadotrophin: What Should We Measure and How? Clinical Chemistry. 2009; 55(11): 1900-1904. doi: 10.1373/clinchem.2009.133595
3. Al-Mahdili HA, Jones GR. High-dose hook effect in six automated human chorionic gonadotrophin assays. Annals of Clinical Biochemistry. 2010; 47: 383-385. doi: 10.1258/acb.2010.090304
4. Greene DN, Petrie MS, Pyle Al, Kamer SM, Grenache DG. Performance characteristics of the Beckman Coulter total βhCG (5th IS) assay. Clinica Chimica Acta. 2015; 439: 61-67. doi: 10.1016/j.cca.2014.09.029
5. The Access Total βhCG (5th IS) [package insert]. California (US). Beckmann Coulter, Inc; 2013.