



## Case Report

# Variant hook effect: A cause of false negative result in point of care testing

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### ABSTRACT

Beta Human Chorionic Gonadotropin ( $\beta$  hCG) assays are sandwich chromatographic immunoassays which give a false negative results at high hCG concentrations and also in the presence of variants of hCG like hyperglycosylated hCG and hCG core fragments. This is imperative in everyday clinical scenario as it delays the diagnosis of pregnancy and its related complications. We have discussed two such cases where in the first case the diagnosis of hydatidiform mole was delayed due hook effect whereas in the second case the diagnosis of GTD was missed because of variant hook effect.

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## 1. Introduction

Beta Human Chorionic Gonadotropin ( $\beta$  hCG) assays are sandwich chromatographic immunoassays, they produce false-negative pregnancy test results in the presence of excessively high antigen concentrations due to a phenomenon known as the “high-dose hook effect,” or due to presence of high concentration of hCG variants known as “variant hook effect.”<sup>1</sup> However, in literature, very few descriptions of the hook effect in gestational trophoblastic diseases (GTDs) have been sighted. This phenomenon can be overcome by diluting the sample, hence decreasing the antigen concentration, leading to a positive test which was initially negative. It is rare to demonstrate ‘hook effect’ in normal pregnancy, mostly it is encountered in GTDs where either the hCG levels are very high to create the prozone phenomenon (which is excessive antigen saturating the antibodies) or due to presence of high concentration of hCG variants like glycosylated hCG or core fragments.

## 2. Case Series

### 2.1. Case 1

A 25 yrs old G4P3L3, with last child birth 3 yrs back presented with 2 months amenorrhea with excessive bleeding for past 3 hours to our emergency. On examination, her pulse was 120/min, her BP was 90/60. She was clinically 4-5 gm%. On local examination her uterus was just palpable and abdomen was soft with no tenderness or rigidity. Her per vaginal examination revealed bulky uterus with os closed with bleeding present. She was not having any ultrasound report and her urine for pregnancy test was negative. Ultrasound done in emergency was inconclusive, it did not show intrauterine pregnancy and showed presence of hyperechoic content likely to be either clots or degenerated fibroid or sub-mucous polyp. Since pregnancy was ruled out, the patient was admitted to the gynecology high dependency unit (HDU) for blood transfusion and stabilization of patient. As soon as the patient was admitted to the HDU, she had a heavy bout of bleeding with passage of grape like vesicles making the diagnosis of molar pregnancy almost certain. Her urine for pregnancy test (UPT) was repeated after diluting the urine with normal saline keeping the ‘hook effect’ in mind, which came out to be positive hence confirming the diagnosis of molar pregnancy and the patient was immediately shifted

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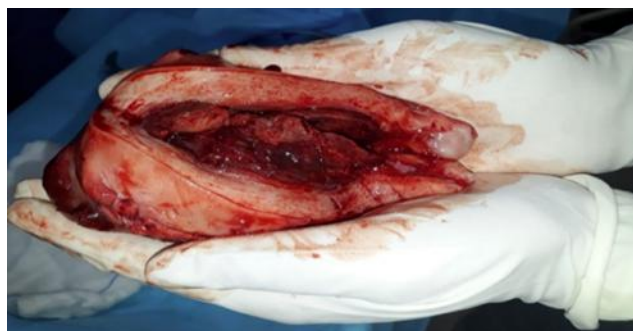
for suction evacuation and blood for serum hCG levels was sent which came out to be 1,20,000. Patient was shifted to operation theater and suction evacuation was performed under general anesthesia. The patient recovered well after suction evacuation and was discharged on 5<sup>th</sup> day after correcting her anemia and investigating her completely for any evidence of metastasis. She was discharged with advice for proper follow up of molar pregnancy. This was our first encounter with the 'hook effect'.

## 2.2. Case 2

A 26 yrs old Para-3 Living-3 Abortion-1 female was referred to our hospital in hypovolemic shock on inotropic support, and was immediately intubated on admission to our HDU. Patients relative gave history of abortion 5 months back at 4 months amenorrhea at some private hospital, following which patient had on and off bleeding for 4 months. Patient underwent suction and evacuation in view of retained product of conceptus twice in the last 4 months but her bleeding still continued. She developed high grade fever on 2<sup>nd</sup> day of last dilatation and evacuation and was started on high grade antibiotics. Her general condition kept deteriorating and bleeding per vaginum continued. Patient was transfused 2 units of blood, started on inotropic support and referred to our hospital. Our first differential diagnosis for this patient was GTD but the urine pregnancy test came out to be negative, still due to high index of suspicion UPT was done with serial dilution of urine which became positive at 1:40. Serum hCG levels were sent and ultrasound of the patient was performed which revealed intraperitoneal collection with signs of rupture. The patient was taken up for emergency laparotomy. Per-operative haemoperitoneum of around 1.5 L noted, with a diffuse infiltrating mass arising out of the uterus invading its serosa. Total hysterectomy was done and specimen was sent for histopathology. Patient was shifted to intensive care unit in the post-operative period. Her serum hCG levels were found to be 1,35,000 and X-ray showed presence of lung lesions suspected to be lung metastasis Unfortunately the patient went into irreversible shock followed by acute renal shutdown on the 2<sup>nd</sup> post-operative day and died on the 3<sup>rd</sup> post-operative day. Her histopathological report of the operated specimen confirmed the diagnosis of choriocarcinoma. [Figures 1 and 2]. This patient was lost due to false negative pregnancy test in previous centres which failed to raise an alarm about GTD.



**Fig. 1:** Total hysterectomy specimen of case 2



**Fig. 2:** Cut section of uterus showing GTD

## 3. Discussion

There are two clear categories of hCG test: 1) intact hCG tests - designed to detect the intact dimer only. 2) Total hCG tests (most commonly used in commercial laboratories), also known as b-hCG tests designed to detect the intact dimer, as well as the free b-subunit, nicked and other variants of hCG. In the present clinical scenario, all variants of hCG are considered to be one by treating doctors who often order an hCG test for pregnancy, GTD, and cancer cases, regardless of the analytical specificity of the test. The Food and Drug Administration (FDA) approves any test that detects regular hCG and gives no importance to the presence of other variants of hCG, including hyperglycosylated hCG [the principal form present in early pregnancy] and free b-subunit, [the only form produced by cancers].<sup>2</sup>

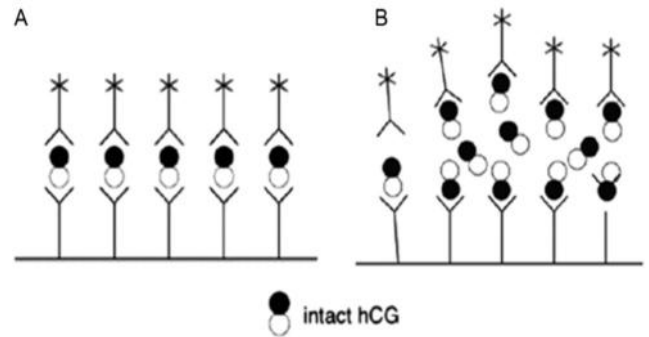
The principle of the modern laboratory hCG immunoassay is based on the sandwich technique whereby a capture antibody binds one site on hCG, immobilizing it and a signaling antibody binding a distant site on hCG, hence labeling the immobilized complex with an enzyme immunoassay (EIA) or with radioactive iodine. The immobilized and labeled complex is then quantified, the radioactive signal from the complex being directly proportional to the amount of complex or concentration of hCG.

### 3.1. Point of care testing

The dual antibody-sandwich techniques are also the basis of all urine POC(point of care) rapid pregnancy tests. The most common type of POC device is made of nitrocellulose and plastic. Urine is dropped with a pipette into one window, and a positive result is shown by a line or a cross in a separate window. No readily available POC tests are sensitive enough for detecting free b-subunit or b-core fragment.<sup>3</sup>

Hook effect' is an important cause of false negative pregnancy test. It can occur in the presence of excessively high intact hCG concentration or presence of variants of hCG like glycosylated hCG or core fragments. Hook effect was first described by Miles in the year 1975 while performing an immunoradiometric assay for ferritin, various other hormones like prolactin, growth hormone, hCG, TSH, gonadotropins and aldosterone have also shown hook effect at high concentration.<sup>1</sup> The High Dose Hook effect leading to false negative results occurs in sandwich immunoassays, when the concentration of the substrate increases above a certain critical value, the system gets saturated and the signal begins to decline. It usually happens at hCG concentration of more than 500000 mIU. To eliminate the false negative results due to Hook effect the sample should be diluted before running the test.<sup>3,4</sup>

A similar but still distinct hook like effect also known as variant hook effect has been reported to occur with certain variants of hCG like hCG-H, nicked hCG hypo or hyperglycosylated hCG, all of which are also known to produce false negative pregnancy test even in the presence of high quantities of hCG in the sample. This occurs when there is a high concentration of these variants in the sample and the antibodies in the routine sandwich immunoassays fail to bind to the variant hCG, hence leading to a negative pregnancy test. In GTDs as mentioned in the cases above, the core fraction of hCG is higher than the intact hCG leading to a false negative pregnancy test.<sup>4</sup> As the high dose hook effect disappears on dilution of urine, the same holds true for variant hook effect which disappears on dilution. Dilution has to be done from 1:10 to 1:100. We take 1cc urine and make it 10 cc by saline and repeat the UPT, we can keep adding 10 cc diluent till we get a positive test or we reach a dilution of 1:100 whichever is before [Figure 4]. Diluting the original sample will dilute the amount of hCG variant that cannot be detected and, hence, free some of the saturated antibodies and give a positive result. Also, the recently introduced DPC immunolite assays, can detect such variants of hCG and help in the diagnosis of GTDs.<sup>2,3</sup>



**Fig. 3:** A): Showing sandwich formation between capture and signal antibody with antigen in between; B): Showing hook effect and inability to form a sandwich, due to saturation of capture and signal antibody



**Fig. 4:** Showing positive hcg urine pregnancy test on diluting the urine of a patient with GTD

## 4. Conclusion

Various clinicians all around the world have reported about 'hook effect'. There are various citations regarding it in the literature. Various clinicians have published like Datti SN,<sup>5</sup> et al. reported a false negative pregnancy test in case of partial mole and choriocarcinoma, also Pang YP et al. reported a false negative test in molar pregnancy.<sup>4</sup> Also similar case reports have been mentioned by Nalini et al. who again reported it in molar pregnancy. Also it has been reported by pathologists in the study by Yadav et al<sup>6</sup> who reported it early pregnancy cases. There are many other case reports throughout the world reporting 'Hook Effect' stressing about its importance in daily testing.

A false negative pregnancy test may have serious consequences and can add to maternal morbidity in cases like hydatiform mole and GTN, as it leads to delay in diagnosis of these life threatening conditions. It should be made a routine practice to test for pregnancy after diluting the urine when there is a strong suspicion of pregnancy or related complications. Also hook effect due to hCG variants should be kept in mind while making a diagnosis.

## 5. Source of Funding

None.

## 6. Conflict of Interest

None.

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