

# The hook effect: pregnancies with persistent negative pregnancy tests

## Case report

Dr. Mustafa Ahmed<sup>1</sup> MD DFM MRCOG Mr.N.Venkat Raman<sup>2</sup> MD DGO FRCOG Mr Singh, M<sup>3</sup> MRCOG Dr. Yunus,Deeba

1- Clinical Fellow Southend UH 2- Consultant - Southend UH 3- Consultant Southend UH 4- Registrar- Southend UH

### Summary

**39 year old lady P 4 was referred to by the community midwife with amenorrhoea for 10 weeks, nausea and vomiting but negative pregnancy test. The lady claimed that all her pregnancy showed negative pregnancy test at that stage. She is known epileptic on carbamazepine 5mg and had fit 2 days before presentation.**

**Transvaginal scan showed an intrauterine sac equivalent to 5 weeks but no fetal pole and small cyst to the left side of the uterus. Hence, she was referred to the EPU for follow up and scan was booked for her in our clinic at what would be equivalent to 8 weeks for reassessment.**

**At the EPU pregnancy test was repeated twice and the result was negative and the lady was reassured and discharged from follow up.**

**However, the lady attended to our clinic appointment as she continued to have severe symptoms of pregnancy and remained to be amenorrhoeic. TVS showed an intrauterine fetus of 8 weeks with positive heart beat. She claimed that she has repeated the pregnancy test after being discharged from the hospital and the test remained to be negative. She had 3 fits since being discharged last time.**

**In addition to her 12 weeks scan, early anomaly scan was booked at 16 and 18 weeks in view of her medication which she continued to use during the first trimester. Moreover, a joint consultant appointment was booked to modify her epilepsy medication for better control. She declined first trimester screening as she was committed to the pregnancy.**

**The pregnancy continued up to term and complicated by moderate pre-eclampsia in the third trimester for which she was offered induction of labour and delivered a healthy baby.**

### Discussion

Persistent negative pregnancy test has been reported in twin pregnancy and this is the first time to be reported in singleton pregnancy.

In this case report, despite the lady's history and TVS findings, she was discharged from follow up in view of persistent negative pregnancy test. That has put her at risk of repeated seizure as the effect of pregnancy on epilepsy is well known, and put the growing fetus at risk of multiple anomalies that can result from anti-epileptic medication.

In addition to the above risks, the hospital would have faced a legal action in case of aggravation of the patient condition or if any AEDs--anomalies has been discovered later in pregnancy.

### Background

Human chorionic gonadotropin is a glycoprotein comprised of  $\alpha$  and  $\beta$  subunits that is produced in the placenta after implantation. The  $\beta$ -subunit of hCG is what is measured in pregnancy tests, as the  $\alpha$  subunit can also be found in other hormones such as luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone. The  $\beta$  subunit exists in several forms, including hyperglycosylated hCG (H-hCG), nicked hCG, free  $\beta$  subunit, the core fragment of  $\beta$ -hCG (hCG- $\beta$ cf), and others (Figure 3). The relative fractional concentrations of these forms change throughout pregnancy. For example, H-hCG is the primary, if not sole form of hCG produced after implantation, and accounts for up to as much as 60% of hCG found at 4 weeks, dropping to < 5% in the second and third trimesters.<sup>[7]</sup> By contrast, hCG- $\beta$ cf is high in mid-pregnancy urine.

Urine and serum hCG concentrations vary widely during pregnancy, with urine concentrations measuring approximately half that of the corresponding serum fractions, depending on the hydration status of the patient. The reported thresholds for detection in pregnancy tests kits are between 15 and 100 mIU/mL. Various pregnancy test kits may not detect certain hCG variants as well as others, and sometimes not at all. One study reported that a majority of urine pregnancy tests had poor sensitivity for H-hCG, which typically is the highest concentrated variant of hCG present in early pregnancy.

### The Hook effect

A known failure mode for pregnancy detection by urine hCG pregnancy tests occurs when hCG is in large excess and no dilution step is performed to wash the excess hCG variant away. The excess hCG form can simultaneously saturate the fixed, solid-phase antibody and the labelled, soluble antibody, preventing sandwich formation (Figure 1). This "hook effect phenomenon" results in absence of a signal, and thus a false-negative result. The paradoxical decrease in the curve plotting an increased signal with increased concentration of antigen gives the appearance of a hook, hence the name. In urine hCG tests, the hook effect is generally not observed until hCG concentrations reach 1,000,000 mIU/mL, as is seen in gestational trophoblastic disease. However, the "hook effect" can also cause false-negative results in normal pregnancies when an excess of hCG is present.

A similar but distinct "hook-like" effect was hypothesized to occur when one of the antibodies in the sandwich configuration is unable to bind a particular hCG variant such as hCG- $\beta$ cf, whereas the other antibody avidly binds this fragment. Under normal conditions, a sandwich can still form when the two antibodies both bind to other hCG variants present, resulting in a positive test. However, when the variant (here, hCG- $\beta$ cf) is in high concentration, it will saturate the antibody that recognizes it to the exclusion of other variants present. Because the other antibody in the sandwich assay does not bind hCG- $\beta$ cf, sandwich formation (both fixed and soluble antibodies binding the hCG variant) cannot occur, resulting in a false-negative test. In early pregnancy, false negatives can result when hCG- $\beta$ cf is in excess, as can happen later in the first trimester. This example is particularly important because hCG- $\beta$ cf is the major hCG beta subunit molecule in the urine from about 5–8 weeks of gestation until term, and accounts for up to 90% of the urine test activity in mid-pregnancy. This same phenomenon presumably can occur with different hCG variants given similar conditions. Although hCG- $\beta$ cf is found almost exclusively in the urine, theoretically this "hook-like" effect can occur in serum as well as urine tests. When a false-negative test is suspected and thought to be a result of the "hook-like" effect, repeating the test after diluting the urine can often resolve the issue. Dilutions for qualitative results are performed usually with normal saline at both 1:2 and 1:5 concentrations.

### Conclusion

False negative pregnancy test may have serious consequences and some avoidable causes of maternal and fetal morbidity and mortality can be missed. Hence, repeating pregnancy test after dilution should be tried in all cases where pregnancy is likely while pregnancy test remains to be negative.

In addition to the dilution method, it will be a good practice to arrange an early follow scan in such cases since some tests may not detect certain variants of hCG.

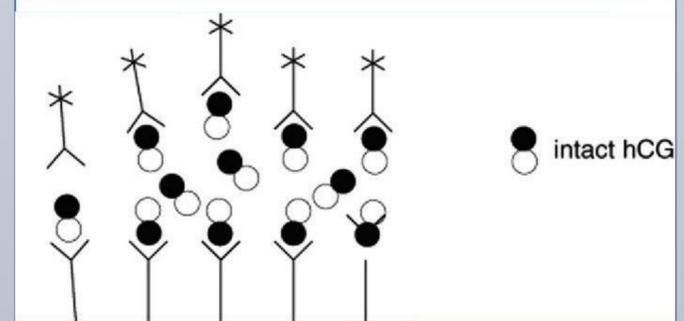


Fig-1

### References

- 1- Brewer C. Failure to diagnose pregnancy as cause of late abortion [Letter]. *Lancet* 1977;1:46.
- 2- Butler SA, Khanlian SA, Cole LA. Detection of early pregnancy forms of human chorionic gonadotropin by home pregnancy test devices. *Clin Chem* 2001;47:2131–6.
- 3- Wilcox AJ, Baird DD, Dunson D, McChesney R, Weinberg CR. Natural limits of pregnancy testing in relation to the expected menstrual period. *JAMA* 2001;286:1759–61.
- 4- Kato Y, Braunstein GD. Beta-core fragment is a major form of immunoreactive urinary chorionic gonadotropin in human pregnancy. *J Clin Endocrinol Metab* 1988;66:1197–201.
- 5- Sturgeon CM, Berger P, Bidart JM, et al. IFCC Working Group on hCG. Differences in recognition of the 1st WHO international reference reagents for hCG-related isoforms by diagnostic immunoassays for human chorionic gonadotropin. *Clin Chem* 2009;55:1484–91.
- 6- Yunus, H Muppala, F Hamer, F Clarke. *Three Consecutive False Negative Pregnancy Tests in a Twin Pregnancy: A Case Report*. The Internet Journal of Gynecology and Obstetrics. 2006 Volume 6 Number 2