

Three Consecutive False Negative Pregnancy Tests in a Twin Pregnancy: A Case Report

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Citation

D 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.

Abstract

We report a case with three consecutive false negative pregnancy tests in a twin pregnancy who presented with severe hyperemesis gravidarum and jaundice. We looked at various possibilities for negative pregnancy tests. Most test kits have one antibody to site β 1 and another to β -CTP or to the β -subunit. β -subunit core fragment, a breakdown product of hCG saturate the β -site and block any hCG from forming a sandwich between β 1 site and the other site, giving false negative result. Dehydration, decreased urinary pH, hepatitis, effect of preservatives and contaminants, ketones and bilirubin, and solubility of various forms of hCG in urine, defective β -HCG biosynthesis and rapid clearance of the hormone from the circulation could have compounded the outcome of the result. It might mislead or delay the diagnosis and could have potential medical implications. Clinical suspicion of pregnancy should be followed up with serum β -hCG and ultrasound.

PUBLICATION NOTE

Both Dr. Yunus and Dr. Muppala contributed equally to this work.

CASE REPORT

A 27-year-old Asian lady was in her third pregnancy. She presented to Gynaecological Assessment Unit with history of 10 weeks amenorrhoea, persistent vomiting and weight loss for 6 weeks, discoloration of eyes for 4 weeks, bruises on palms and dark coloured urine for nearly 2 weeks. On physical examination she looked jaundiced and moderately dehydrated. Vital signs were: temperature 36.5°C, blood pressure 144/84 mm Hg and heart rate of 128 beats/min. Abdominal examination unremarkable. Urine analysis showed ketones +++, blood ++, glucose + and pregnancy test was negative. Since signs and symptoms of pregnancy were present, repeat urine pregnancy tests performed twice but showed no positive line. In view of highly suspicious of our patient being pregnant clinically, abdominal ultrasound performed and revealed to be twin pregnancy corresponding to 9 weeks + 5 days. She was admitted in view of hyperemesis gravidarum and jaundice.

Blood samples were sent to laboratory. She was given IV fluids in the form of normal saline, multiple antiemetics (metoclopramide, cyclizine, ondansetron) in view of severity of vomiting, thiamine, TEDS and clexane. Laboratory findings were: Hb 139 g/L, WCC 11.9×10^9 /L, Platelets

297×10^9 /L, Serum β -hCG of 459139 IU/L, Total bilirubin 64 μ mol/L (3-28), GGT 79 IU/L (0-60), ALT 267 IU/L (0-50), Free T3 13.8 pmol/L (3.2-7.5), Free T4 87.0 pmol/L (9-22), TSH <0.03 mIU/L (0.25-5.00), Na⁺ 133 mmol/L (135-145), K⁺ 2.7 mmol/L (3.6-5.4), normal serum urea and creatinine levels. The symptoms of nausea and vomiting were slowly settling and she was able to tolerate oral fluids and solid diet in small amounts. But in view of persisting jaundice, tachycardia and deranged LFTs and TFTs a medical opinion was requested. The only real clinical manifestation of thyrotoxicosis is her tachycardia and slight tremors. Her abnormal TFTs were managed conservatively.

Screening tests for Infectious mononucleosis, Hepatitis A, B, C, anti-mitochondrial, anti-smooth muscle antibodies, α 1 antitrypsin, ferritin and clotting profile were requested for and all came negative. Our further plan were to continue same conservative management and to repeat LFTs biweekly and TFTs once weekly as our patient was improving clinically. Patient was discharged on 7th day following clinical improvement and normal biochemical parameters. Total bilirubin 18 μ mol/L, GGT 62 IU/L, ALT 216 IU/L, Free T3 5.6 pmol/L, Free T4 29.1 pmol/L, TSH <0.03 mIU/L. She had dating scan around 12-13 weeks of gestation corresponding to her dates, reported as dichorionic and diamniotic twins and normal anomaly scan at 20 weeks of gestation. She continued to have midwifery and consultant shared, antenatal care. By the time this case report is

submitted our patient had semi-elective caesarean section in early labour and gave birth to healthy twin babies at 38+ weeks of gestation.

DISCUSSION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone with an α and β subunit joined non-covalently. The free beta subunit of hCG differs from other pituitary gonadotrophins in that it has a 30 amino acid tail piece at the COOH terminus. Free beta subunits are degraded by macrophage enzymes in the kidney to make a beta subunit core fragment, which is primarily detected in urine samples. This hormone is produced by developing placenta, and is significantly elevated above normal in twin pregnancy within wide variation compared to normal gestation.

Any women of reproductive age who comes to emergency department or gynaecological assessment unit, urine qualitative assay for hCG is performed to know whether they are pregnant or not and it is the standard of care we follow and that clinical decisions are based on this screening test. Our Gynaecological department uses one of the several commercial urine pregnancy tests available in the market, to detect normal pregnancy from a urine sample. This is a qualitative, two site sandwich immunoassay for the determination of hCG in urine. The test utilises monoclonal antibody reagents to selectively detect elevated levels of hCG in urine. We followed the manufacturer instructions with regarding to testing and interpretation of results. Procedural error or deterioration of test reagent negated by the presence of pink-coloured line in the control region.

From our point of view, one or all of the reasons stated below could have caused false negative results in our case.

1. There are multiple forms of hCG in maternal plasma or urine. Some of these arise as the result of enzymatic degradation; and others are accounted for by modifications during the normal cellular sequence of synthesis/processing of the hCG molecule. The multiple forms of hCG vary enormously in bioactivity and immunoreactivity.¹ Serum β -hCG levels are excessively elevated for gestational age in twin pregnancies. Since this being a dichorionic-diamniotic twin pregnancy, there is a possibility that heterogeneous molecules of hCG^{2,3,4} were produced from two placentas, and which failed to produce immunoreactivity with the pregnancy kit.^{2,5}

2. In the interference testing of this assay, the outcome was not affected by bilirubin concentration of 1mg/dl. Since the

patient is jaundiced, concentration of bilirubin in urine was much higher that could have affected the outcome of the result.

3. Dehydration leads to concentrated and less urine production which in turn might contain high concentration of hCG.^{6,7} So, if very high levels of hCG are suspected, the test should then be repeated with the diluted specimen, 1 in 10 or 100.

4. Since dehydration leads to concentrated and acidic urine, that is, if the pH of the urine is below 5, then the test might theoretically affect the result. This kit is tested for the effect of urine pH from 5-9 with hCG concentrations at 20 mIU/mL and that did not affect the outcome of the result. Also the solubility of different variants of hCG in acidic urine is a factor that needs to be taken into account when considering false negative results. For example, the Hyperglycosylated hCG, which predominates in early pregnancy, is more acidic and its solubility is enhanced in acidic urine.⁸ Also, ketones which are acidic could have made hCG more soluble in urine.

5. The renal clearance of hCG accounts for 30% of metabolic clearance and the remainder is cleared by metabolism in liver and kidney.¹ If the metabolism of hCG is affected in hepatitis as in this case, all of the serum hCG might be metabolising in kidney and excreted in the urine, leading to high dose hook effect.⁴

6. Defective β -hCG biosynthesis and rapid clearance of the hormone from the circulation could also be a possibility in very rare cases.⁹

According to Prof. Larry Cole, the Friedman and Howard Distinguished Professor of Ob-Gynae, who is an authority on β -hCG and runs a HCG Reference Service at the University of Mexico, USA; the 99% accuracy quoted by manufacturer means that it detected at least 99% of high (>100mIU/mL) hCG standards. It has zero relationship to what percent of pregnancies it detects. Prof. Larry Cole has an unpublished experience that most tests with multiple manufacturers test kits will present a hook effect problem at hCG levels >200000 mIU/mL in urine. This he has seen many times in his practice of research and has two simple answers. One, most devices have one antibody to site β 1 and another to β -CTP or to the α -subunit. The principal hCG-related ingredient in urine is " β -subunit core fragment", a small break down product of hCG. This can account for up to 99% of the immunoreactivity. β -core fragment can saturate

the β -site, but not the second site. This will block any hCG from forming a sandwich between the β 1 site and the other site, and lead to a negative result known as “hook effect” or “prozone phenomenon”. Two, the manufacturers urine pregnancy test kit has an upper limit of 600000 which is based upon a standard that is predominantly β -core fragment.¹⁰

Hook effect in itself is an infrequent event leading to false negative results and can be overcome by dilution of urine sample.¹¹ False negative pregnancy tests were reported in ectopic pregnancy, triplets, cancer and trophoblastic diseases but never in an twin pregnancy and that these incidents are not unusually rare compared to the wide usage of pregnancy tests in home, emergency departments and laboratories. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made after pooling together all relevant clinical and laboratory findings. False negative results even if they are extremely rare, may mislead or result in a delayed diagnosis¹¹ and improper follow up or could have potential medical implications following mismanagement. Negative or inconclusive results in patients suspected of pregnancy should be further evaluated by serum quantification of hCG₂ and ultrasonography as demonstrated in this case. Finally, clinicians should understand the possibility of inaccurate results¹¹ and women should be notified of the potential for false negative or false positive results where possible.

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