Case Report:
An Unusual Cause of Elevated Serum Total βhCG

Carl L Buckner,1 Lisa Wilson,2 and Christine N. Papadea1
1Department of Pathology & Laboratory Medicine, and 2Department of Obstetrics & Gynecology, Medical University of South Carolina, Charleston, South Carolina

Abstract. Human chorionic gonadotropin (hCG), a heterodimeric hormone consisting of an alpha (α) and a beta (β) subunit, is used as a marker for the diagnosis of pregnancy, congenital defects, and choriocarcinoma. After excluding the common causes of elevated serum hCG, laboratory identification of false-positive or true results assists in guiding clinical management. Options include testing urine for hCG, serum for heterophile antibodies, and serum hCG by different immunoassays. We report the case of a non-pregnant patient with chronic renal failure who had a positive urine hCG test, an elevated serum hCG level by two different assays but normal by a third assay, and persistently elevated serum hCG levels after ruling out the likelihood of heterophile antibodies. The discrepancies were explained by the patient’s impaired renal clearance and the molecular forms of hCG that were measured by each assay. This case illustrates the importance of the laboratory’s role in understanding the causes of elevated serum hCG.

Keywords: hCG, hemodialysis, heterophile antibodies, immunoassays, pituitary hormones

Introduction

Human chorionic gonadotropin (hCG) is a heterodimeric hormone consisting of an α (14.5 kD) and a β (22.2 kD) subunit. The α-subunit of hCG is the same as in other pituitary hormones—luteinizing hormone, follicle stimulating hormone, and thyroid stimulating hormone—whereas the β-subunit is unique and confers specific biological functions for each hormone. hCG is produced in small amounts by the pituitary and other organs, including the testis, liver, colon, and in much larger amounts by placental trophoblast and by malignancies such as hydatidiform mole, choriocarcinoma, and germ cell tumors [1]. Therefore, hCG is a useful clinical marker for detecting and monitoring various physiologic and pathologic conditions. Depending on its source or the condition, hCG may be present in serum in different forms such as the intact active dimer, free β-subunit, and various modified forms of the β-subunit. Several commercial immunoassays are available to measure hCG in serum and urine. They differ with respect to the specificity of reagent antibodies that determine the molecular form(s) of hCG detected by each assay. The configurations designed as two-site (sandwich) immunometric assays are susceptible to interferences by heterophile antibodies, which may cause false results and harmful consequences to the patient. Laboratories have attempted to detect this source of interference in various ways, such as testing the patient’s serum by other hCG immunoassays, adding a heterophile antibody blocking reagent to the serum and re-testing, and by other approaches. After interfering heterophile antibodies have been ruled out as the cause of a false-positive serum hCG test, and pregnancy and malignancy have been eliminated as common causes of a positive result, a true positive may also be explained by an uncommon but interesting cause, as described in the present case.
Case Report.

The patient is a 35-yr-old African-American woman who presented in August 2005 with a serum hCG level of 290 mIU/mL (normal <5 mIU/mL). Establishing a date of conception was impossible due to irregular menses. An ultrasound examination was performed on the day of hospital admission, but no gestational sac was detected. An endometrial stripe of 46 mm was seen, along with a 3 x 2 cm right adnexa and 5 x 5 x 3 cm left complex adnexa with multiple septations. The differential diagnosis included missed abortion, ectopic pregnancy, hydatidiform mole, malignancy, and pituitary abnormality. A cervical dilatation and uterine curettage showed no chorionic villi or fetal parts and no evidence of malignancy, which ruled-out missed abortion and hydatidiform mole. An exploratory laparotomy was performed 2 weeks later, which showed bilateral hydrosalpinx, marked intra-abdominal adhesions, and bilateral complex adnexal masses. The patient underwent bilateral salpingectomy and right oophorectomy. Microscopic examination revealed bilateral hydrosalpinx, a benign ovary, and no evidence of ectopic pregnancy. Serial measurements of serum hCG remained moderately elevated after the surgery (Table 1).

The patient's previous medical history is significant for oligouria secondary to end-stage renal disease (ESRD), for which she was initially treated with peritoneal dialysis and then hemodialysis during the previous 7 years. She also has a history of hypertension, gastroesophageal reflux disease, and obesity. Her surgical history includes a left corneal transplant due to bilateral keratoconus. Her obstetric and gynecologic history is extensive and includes a spontaneous vaginal delivery with her first pregnancy. She received no prenatal care and delivered at home at 8-mo gestation. The infant died shortly thereafter. She had received Depo-Provera for contraception, but the last injection was 2 to 3 yr previous. Her last Pap smear was >10 yr ago and she had been treated for syphilis 20 yr ago.

Methods

All serum samples for hCG in this patient were tested in the main laboratory of our hospital by a two-site immunochemiluminometric assay (ICMA) using an automated analyzer, (ADVIA Centaur Total hCG assay, Bayer Diagnostics, Tarrytown, NY). Selected samples from the patient that were available in sufficient amount were also tested for hCG by

<table>
<thead>
<tr>
<th>Date</th>
<th>Serum hCG, mIU/ml</th>
<th>Serum creatinine, mg/dl</th>
<th>Urine hCG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICMAa</td>
<td>ICMA</td>
<td>IEMA</td>
</tr>
<tr>
<td>8/22</td>
<td>290</td>
<td>150 (1:2)</td>
<td>78 (1:4)</td>
</tr>
<tr>
<td>9/3</td>
<td>285</td>
<td>456</td>
<td></td>
</tr>
<tr>
<td>9/4</td>
<td>232</td>
<td>245</td>
<td></td>
</tr>
<tr>
<td>9/5</td>
<td>197</td>
<td>202</td>
<td></td>
</tr>
<tr>
<td>9/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/7</td>
<td>238</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/8</td>
<td>231</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/15</td>
<td>250</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Summary of the patient's laboratory results.a

<table>
<thead>
<tr>
<th>Date</th>
<th>Serum hCG, mIU/ml</th>
<th>Serum creatinine, mg/dl</th>
<th>Urine hCG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICMAa</td>
<td>ICMA</td>
<td>IEMA</td>
</tr>
<tr>
<td>8/22</td>
<td>290</td>
<td>150 (1:2)</td>
<td>78 (1:4)</td>
</tr>
<tr>
<td>9/3</td>
<td>285</td>
<td>456</td>
<td></td>
</tr>
<tr>
<td>9/4</td>
<td>232</td>
<td>245</td>
<td></td>
</tr>
<tr>
<td>9/5</td>
<td>197</td>
<td>202</td>
<td></td>
</tr>
<tr>
<td>9/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/7</td>
<td>238</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/8</td>
<td>231</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/15</td>
<td>250</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Reference ranges: serum hCG, all methods: 0-4 mIU/mL; serum creatinine, 0.6-1.1 mg/mL; urine hCG, negative.
- aThe hCG urine test measures hCG qualitatively with a single monoclonal antibody specific for the β-subunit of hCG. It is referenced to the WHO 4th International Standard (IS) 75/589.
- bICMA, ADVIA Centaur Total hCG, a two-site immunochemiluminometric assay constructed with a polyclonal goat anti-hCG antibody and a monoclonal anti-β-hCG antibody. Molecular forms detected include intact whole hCG, free β-hCG subunit, nicked whole hCG, C-terminal peptide of the β-hCG subunit. The assay is calibrated against WHO 3rd IS 75/537.
- cIEMA, AxSYM Total β-hCG, a two-site immunoenzymetric assay constructed with a polyclonal goat anti-β-hCG antibody and a monoclonal anti-β-hCG antibody to measure the free β-subunit and the β-subunit of the intact hCG. Molecular forms detected include intact whole hCG, free β-hCG subunit, nicked whole hCG, nicked free β-hCG subunit. The assay is calibrated against WHO 4th IS 75/589.
- dECIA, Elecsys hCG STAT, an electrochemiluminescence immunoassay, configured with 2 monoclonal hCG-specific antibodies. Only the intact whole hCG is detected. The assay is calibrated against WHO 3rd IS 75/537.
- eHBT, heterophile antibody blocking test. This method uses a proprietary binding agent to inactivate heterophile antibodies (“pretreat”) before the serum is tested for total hCG. If heterophile antibodies are present in the serum, the hCG concentration in the treated sample typically would be lower than in the original.
- fSerum dilutions, in parentheses, are 2-fold and 4-fold in a serum-based, hCG-free diluent provided by the manufacturer. After multiplying by the dilution factors the corrected concentrations are 300 and 312 mIU/mL, respectively.
our laboratory using two automated two-site immunometric methods, considered ancillary to the primary method. These included the AxSYM Total β-hCG immunoenzymetric assay (IEMA; Abbott Diagnostics, Abbott Park, IL) and the Roche Elecsys 2010 hCG STAT electrochemiluminescence immunoassay (ECIA; Roche Diagnostics, Indianapolis, IN). The specific molecular forms of hCG measured, the reagent antibodies, the HCG calibrators, and the signal ligands vary among these immunoassays (Table 1).

For dilutional studies, we used the diluent (bovine and goat serum with sodium azide as preservative) provided for the AxSYM Total β-hCG IEMA kit. The patient’s urine was tested by the QuickVue One-Step hCG Urine Test (Quidel Corp., San Diego, CA), the qualitative method used routinely by our main laboratory for pregnancy testing. It detects hCG >25 mIU/mL with a single monoclonal antibody having specificity for the β25 mIU/mL with a single monoclonal antibody having specificity for the β-subunit of hCG. The heterophile antibody blocking test was from Scantibodies Laboratory, Inc. (Santee, CA). This test consists of adding the suspected serum to a tube coated with a proprietary binding agent that adsorbs heterophile antibodies and rheumatoid factor if they are present in the serum. If the hCG concentration in the treated serum is less than in untreated serum, the presence of heterophile antibodies is suspected. The results for all tests described were obtained in accredited hospital laboratories according to standard operating procedures, established quality control guidelines, and the instructions of the manufacturers of the respective kit methods.

**Results**

The patient’s results for serum creatinine, serum hCG, and urine hCG are shown in Table 1. The elevated serum creatinine levels reflect the patient’s end-stage renal disease. The original serum of 8/22/05 was found to have an elevated hCG (290 mIU/ml) and was subsequently tested with serial dilutions (1:2 and 1:4) to determine the proportionality of the results. The concentrations being proportional to the dilution factors mitigated the likelihood of heterophile antibodies. Three other samples originally tested by ICMA (on 9/3, 9/4, and 9/5) were re-tested by 1 of 2 other immunoassays, IEMA or ECIA. The IEMA confirmed the elevated result by ICMA; however the ECIA values were strikingly lower at <5 mIU/ml. The hCG concentrations of the two samples (9/4 and 9/5) treated with the heterophile blocking reagent were similar to the original values (within the 95% confidence interval of the assay), and the patient’s urine (9/7) was positive for hCG, which both weighed against the likelihood of heterophile antibodies.

**Discussion**

This case illustrates a perplexing discrepancy between clinical observations and laboratory values. A basic understanding of the analyte in question, the assays used to measure it, and laboratory approaches to reconcile the dichotomy of analytical results will be reviewed briefly.

hCG exists in many forms including the whole molecule, nicked (enzymatically cleaved), hyperglycosylated, free subunits, β core fragment, β C-terminal peptide, and β-subunit without its C-terminal peptide. When the β-subunit becomes nicked between its amino acids 47 and 48, the β-subunit dissociates from the α-subunit and is degraded to the β core fragment. This β core fragment is the final degradation product of the β-subunit and may be the only form secreted in molar pregnancy or malignancy. The whole molecule is the major form found in serum and the β core fragment is the major form in urine. Many commercial immunoassays are available to detect hCG. They differ with respect to (a) the antibody reactivity for the molecular forms they detect (ie, non-nicked only, nicked and non-nicked, non-nicked and free β-subunit, etc), (b) the number of different forms of the molecule in the calibrator preparations, and (c) the assay construction with one or more antibodies [2]. Thus, we selected the 3 serum hCG assays available in our laboratories for the various molecular forms they measure, and not for the different detection signals they employ.

Most commercial hCG immunoassays are based on the sandwich principle, which uses two antibodies. The first is a monoclonal anti-hCG antibody fixed to a solid phase to capture the hCG antigen in the sample. The addition of the second monoclonal or polyclonal anti-hCG antibody conjugated with a signal agent is directed against a different site on the antigen to form a sandwich reaction with the hCG. The amount of signal from the bound conjugate is compared to a calibration curve to determine the concentration of hCG in the sample. A commonly used immunoassay for urine hCG designed with only one antibody is the qualitative urine test constructed with an antibody that reacts with the β core fragment. All immunoassays, regardless of their specificity and antibody
configuration, are susceptible to interference from nonspecific sources such as heterophile antibodies.

Heterophile antibodies are human antibodies against animal antibodies and come in 2 varieties. The first type is rare, is specific to animal antibodies, and is formed after exposure to antibodies such as the murine monoclonals used for diagnostic or therapeutic purposes. The second, more common, type consists of nonspecific, low-affinity antibodies such as autoantibodies and antibodies formed after exposure to animal proteins (dietary, environmental contacts, vaccines, and imaging agents).

Heterophile antibodies interfere by binding to the primary and secondary anti-human antibodies in the assays. By binding and bridging these antibodies, they effectively form false sandwiches and generate a false positive signal [3]. Cole and Khanlian [4] reported 61 cases of false positive results due to heterophile antibodies. The patients’ only symptom was a positive serum hCG test. They were all evaluated similarly for normal pregnancy, ectopic pregnancy, and hydatidiform mole, and they were assumed to have choriocarcinoma. Most of these patients underwent unnecessary treatment with chemotherapy and/or major surgery [4]. Rotmensch and Cole [5] published a similar study that involved 12 women, most of whom were treated for suspected choriocarcinoma with surgery or radiation [5]. In all 12 cases, the presence of heterophile antibodies was determined to be the cause of falsely elevated levels of serum hCG.

In order to avoid false positive results due to heterophile antibodies or other interferences, 4 common strategies have been used. The first is to re-test the sample with at least one other method. The second is to conduct a simple qualitative urine test for hCG. Heterophile antibodies, with a molecular weight of approximately 150 kD, are too large to be filtered by the glomerulus, so they should not be present in the patient’s urine and cannot interfere with the result. A third strategy is to employ a heterophile antibody blocking test. A previously reported retrospective and prospective study described 11 false positive βhCG results that were corrected into the normal range or considerably reduced after use of a heterophile blocking reagent [6]. The fourth strategy is to perform dilution studies. The patient’s sample is serially diluted in an hCG-free, protein matrix preparation and the hCG is measured in the original and the diluted samples. Non-parallel results, i.e., the concentrations are not proportional to the dilution factors, indicate the presence of an interference. Some laboratories assay all hCG samples in duplicate at different dilutions to help rule out a heterophile antibody; however this may not be cost-efficient for most laboratories. It should be noted that results from different strategies may not produce complementary results [7].

After detecting elevated serum hCG with ICMA, we followed the first strategy by re-testing one sample (9/3) with IEMA and two other samples (9/4, 9/5) with ECIA. The elevated results by IEMA were consistent with those by ICMA. (The values obtained by the two methods were not expected to be numerically equivalent, as these are different commercial assays.) The samples were then retested by a third method, ECIA, in an effort to confirm our findings. This method uses a different set of antibodies that detect the intact hCG molecule only, but it should give a result similar to the other methods if the patient has a true elevated hCG. Results by ECIA contradicted the findings of the first 2 methods, which led us to suspect the presence of a heterophile antibody.

To rule-out a false positive result due to heterophile antibodies, we proceeded with three other strategies and found: (a) the patient’s urine was positive for hCG, indicating the absence of heterophile antibodies; (b) the hCG concentrations in serum did not decrease after treating 2 samples with a heterophile blocking reagent; and (c) a dilutional study of the serum with an hCG level of 290 mIU/ml showed results that paralleled the dilutions. These findings strongly suggested the absence of heterophile antibodies, so we concluded that the patient’s serum hCG results were true positives.

At this point, the patient’s previous medical history was reexamined and a reasonable explanation of our findings was discovered. The patient had been treated for end-stage renal disease with hemodialysis for the past 6.5 yr. The significance of this fact was found in a study published by Schwartz et al [8] in 1985 in which serum βhCG levels were followed in 18 women and 4 men on
chronic hemodialysis. Of this cohort, only the women had elevated levels, some with a 10-fold increase over the upper limit of the normal range. The levels increased after the patients underwent dialysis and the levels became normalized in 2 women after renal transplantation [8]. Since hCG is not produced exclusively by trophoblasts, other tissues, notably the pituitary gland, can provide a constant source of intact whole hCG molecule and the free β-subunit at a slow rate [9].

The sieving coefficient of our patient’s dialyzer is 0.63 for an 11.8 kD protein (β2-microglobulin) and 0.3 for a 30 kD plasma protein (unspecified) according to manufacturer’s information (Polyflux 24R, Gambro Renal Products, Lakewood, CO). Considering that the molecular sizes of intact hCG (36.7 kD) and its β-subunit (22.2 kD) are larger than the 2 marker proteins mentioned above, we speculate that a significant proportion of the hCG molecules would not be removed from the patient’s circulation by a dialysis treatment and therefore the serum levels of these molecules would slowly increase over time.

Chronic hemodialysis patients have also been shown to have elevated serum levels of the α-subunit of hCG and other pituitary hormones, and pituitary releasing hormones. Several explanations of this phenomenon have been suggested: reduced metabolism and clearance; increased production due to dysregulation; and changes secondary to uremia [10-13]. These mechanisms may also account for elevated serum βhCG levels in such patients.

A final question to be answered is the reason for the large discrepancy between the serum hCG measurements (Table 1). As mentioned above, various assays for hCG differ in the forms of the molecules detected and the configuration of the assays themselves. The ICMA and IEMA methods are designed to detect total serum hCG in many of its forms. The ECIA method detects only the whole intact hCG molecule. Given the extensive negative investigations of our patient and the laboratory evidence accumulated, we hypothesize that she is secreting and retaining a constant low level of hCG in a form other than the whole intact molecule. As described above, the pituitary gland is a possible source of the free βhCG subunit, which would be detected by the ICMA and IEMA methods but would be undetectable by the ECIA method. Although we had an inadequate amount of serum sample left to test our hypothesis, we hope to obtain additional samples from the patient during a follow-up visit to challenge our suspicions by referring the serum for hCG testing by specialized laboratory tests [2] that are not available in our hospital laboratories.

To our knowledge, elevated serum hCG has been reported infrequently in hemodialysis patients [8]. The incidence of kidney failure treatment in the United States has increased in recent years [14], a trend that leads us to surmise that elevated hCG associated with ESRD, such as in our patient, may be more common than generally recognized.

In summary, there are many sources of hCG and reasons for elevated levels, including both benign and pathologic. Due to the molecular heterogeneity of hCG, it is important to know which form(s) are detected by the specific diagnostic assay used by the laboratory. In patients with elevated hCG levels that are inconsistent with clinical findings, the laboratory should assist the clinician by investigating the unexpected test results by a variety of confirmatory and exclusionary laboratory strategies to avoid harmful mismanagement of the patient.

Acknowledgments

The authors thank William Goodnight, M.D., and N. Brent Hamilton, M.D., for valuable advice and assistance.

References

5. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with


