Use of Beta-Human Chorionic Gonadotropin in Gestational Aging*

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ABSTRACT

Quantitative radioimmunoassay (RIA) of the beta chain of human chorionic gonadotropin (B-hCG) in serum has been used to evaluate the gestational status of 99 normal early pregnancies in contrast to 29 ectopic, threatened, aborted and/or terminated cases. Quantitative measurement of serum B-hCG-RIA standardized against the second international standard (2dIS) accurately established age of normal pregnancies in utero up to but not after three weeks postconception and with an accuracy of plus or minus four days between the third and eighth week of gestation. Quantitative urinary hCG-RIA standardized against the 2dIS were not useful for gestational aging. Useful serum hCG-RIA were identically linear and parallel with the 2dIS, had negligible crossreactivity with LH, FSH and/or TSH, and had low nonspecific binding. Of 13 hCG-RIA evaluated, only assays having these latter characteristics were able to detect ectopic pregnancies, spontaneous abortions, and/or threatened pregnancies with up to 90 percent accuracy. However, some assays not standardized to the 2dIS gave over 200 percent error in hCG serum values. Thus, correct choice of quantitative B-hCG reagents is necessary for early pregnancy assessment.

Introduction

Pregnancy testing in clinical laboratories using quantitative radioimmunoassays (RIA) for human chorionic gonado-

tropin (hCG) has been revolutionized since the emergence of a specific hCG-RIA.20 This assay utilized an antiserum for the beta subunit of hCG which did not react with the alpha chains of leuteinizing hormone (LH), follicle stimulating hormone (FSH), or thyroid stimulating hormone (TSH) nor hormologous portions of their B-chains. Thus, specific

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measurement of B-hCG has become synonymous with the measurement of serum hCG. Exceptions to this concept exist when urine, ascites, or inflammatory exudates are used which may contain fragments or free chains of hCG. Many, but not all commercially available hCG-RIA have become more highly specific and sensitive while the use of hCG assays in determining normal gestational aging has become more complicated. In this same period of time, the evolution of new sexual mores related to highly effective means for pregnancy planning has resulted in increased numbers of ectopic pregnancies, patients with pelvic inflammatory disease and women undergoing pregnancy induction, or in vitro fertilization procedures. This has resulted in an enhanced awareness of early pregnancy.

The use of quantitative assays for the purpose of gestational aging to aid in establishing fetal age in utero is not new. The ligand assay of hCG, human placental lactogen, and pregnancy-specific B-glycoprotein have been related to gestational age 10 days to four weeks after the last menstrual period up to parturition. This study differs in that pregnancy inductions, fertility studies, and rape cases have allowed us the opportunity to establish early serum pregnancy hCG values beginning within the first week of conception. However, difficulties with the use of different commercially available B-hCG kits became known.

Most of the kits utilized in this study claimed to have been calibrated with the Second International Standard for hCG (2dIS). Historically, the 2dIS was produced in 1964 to replace the First International Standard (1stIS) of 1938. It contained 5300 IU as measured by the mouse uterine bioassay and 2.5 mg of hCG. Thus, approximately 0.48 ng hCG, its fragments and/or subunits are found per mIU in the 2dIS. However, the immunologic equivalents will vary according to each manufacturer's choice of antibody(ies), labeling technology, etc. As the 2dIS "runs out", more manufacturers will have to use the First International Reference Protein [1st IRP] formulated in 1975. While the biologic unitage of these preparations is similar, the 1st IRP contains a higher immunologic activity. To correlate the 1st IRP with the 2dIS in w/v, a factor of 5/9 can be applied to obtain a working approximation. However, when an attempt was made by us to establish serum hCG parameters, it was first discovered that certain assays lacked sensitivity and others gave discordant hCG values. When the 2dIS was obtained to calibrate the most sensitive assays for optimal use in early pregnancy detection, pregnancy pools used were standardized with the 2dIS. Thus, to establish truly meaningful B-hCG levels relative to gestational age, 13 commercially available hCG-radioimmunoassays (RIA) were investigated. The use of quantitative urinary hCG-RIA for gestational aging has also been studied, using normal urine in the standard curve and normal serum in the urinary samples to assess the use of any one urinary hCG relative to gestational staging. The normal serum of urinary B-hCG values have been related to ectopic or threatened pregnancies.

Methods and Materials

Patients were entered into this study from the Department of Obstetrics and Gynecology Clinics at New England Medical Center or Saint Margaret's Hospital in Boston, MA. Simultaneous samples of both urine and serum were obtained from pregnant women with their permission. First morning urinary samples were brought into the hospital while the serum was obtained at the clinic on the same day. The date of the last menstrual cycle, dates of sexual
intercourse with partner, or known ovulation inductions were used to establish the gestational age of each sample. Only samples which were obtained from patients with clearly determined gestational histories were used in this study.

Normal pregnancies were determined by clinical examination and a history of the last menstrual period; this was followed by a normal gestation and delivery. Ultrasound, laparoscopy, surgical pathology, and other tests were used only when clinical signs as well as serial B-hCG values indicated an abnormal pregnancy was occurring.

Radioimmunoassay (RIA) kits for the quantitation of serum B-hCG were kindly supplied by each manufacturer. These assays which had been standardized against by 2dIS were run with new isotope in duplicate on two occasions by two different technicians. The data were then reduced according to each manufacturer's directions. Each run included their standard curve, luteinizing hormone (LH) menopausal serum controls which contained approximately 100 mIU as determined by LH-RIA, industry controls, New England Medical Center controls, a male serum control, and a pregnancy serum diluted into the curve with male serum which had been standardized against the 2dIS.

Dilutions from the 2dIS were established by diluting a 100 mIU standard with male serum and a 1/175 dilution in male serum, incubating overnight at 0 to 4°C, centrifuging at 40,000 x g, and diluting with male serum to form hCG-containing pools with ranges at 2.7 to 3.0, 5.0 to 9.0, 18 to 21, 35 to 40, 53 to 64, or 80 to 91 mIU/ml as established on three different occasions with a manufacturer's kit with recoveries identical to the established 2dIS standards. All 13 hCG-RIA kits were calibrated using these known hCG pools with data reduction in accordance with each manufacturer's directions. Quantitative urinary B-hCG values were established by adding 25 µl of male urine to each point of a B-hCG standard curve. Conversely, 100 µl of male hCG-free serum were added to 25 µl of the unknown female sample which was to be assayed. Since the Mallinckrodt kits used for the urinary assays utilized a serum base, no matrix defects were found in the assays.
Female hCG-containing urines were serially diluted with male urine so that values were obtained in the most linear part of the standard curve. These urinary hCG values exhibited parallelism and quantitative recovery with the standard curve when compared with samples spiked with purified urinary hCG. Urinary hCG-values were read off the curve and multiplied by an appropriate dilution factor.

Results

The relationship between quantitative urinary hCG levels and week in utero shown in table I indicates that any one urinary hCG value cannot be a reliable tool for gestational aging. Ranges for urinary hCG overlap up to four weeks. Since some samples had undetectible hCG, 24 hour collections and/or concentration of the urines might have to be applied in certain cases to prevent false negative values.

The quantitation of maternal serum hCG by B-hCG RIA relative to fetal week in utero is shown in table II and figure 1. These data indicate a high probability of assigning gestational status up to the third week of pregnancy. Between the third and ninth week of gestation, a relative week in utero can be estimated with an accuracy of one week. The abnormal pregnancies shown in figure 1 and table IV have an 89.7 percent probability of being pinpointed by a significantly low circulating hCG value. Use of a second hCG determination within four days or serial determinations are useful when a low but normal hCG value is found but clinical suspicion of an ectopic pregnancy exists. Flat or minimally increasing hCG serial determinations help confirm the diagnosis of ectopic pregnancies.

However, correctly quantitating hCG levels relative to the 2dIS will be important if false negative values are to be avoided and accurate gestational ages are to be assigned. In table III, true hCG recoveries and linearity can be assessed as well as low and high end sensitivity for these quantitative B-hCG RIA. Any deviations from linearity shown at either end of any curve or recovery values not in line with the 2dIS indicate where unreliable or indeterminate hCG values will be found.

Discussion

At any one time during the 19 months of this study, between 20 to 30 percent of the positive quantitative B-hCG assays were obtained from women with abnor-
nancy, and emergency cases involving pelvic pain and/or bleeding symptomatic of spontaneous abortions, ectopic pregnancies and/or threatened pregnancies.10,16–17

In table I it is clearly shown that quantitative urinary hCG values as measured by B-hCG RIA were not useful for pregnancy evaluation and/or gestational aging owing to overlapping ranges of four to five weeks. These data may be explained by differential urinary output owing to varying fluid intake or diuretic control. Since several manufacturers have considered producing quantitative urinary hCG kits, these negative data are of current interest. Urinary hCG tests should remain semi-quantitative. Monoclonal antibodies may not be useful in measuring urinary hCG in early pregnancies. This is due to the fact that whole hCG molecules must be present for these reagents to give accurate quantitation of urinary hCG. Use of purified urinary

<table>
<thead>
<tr>
<th>Manufacturer of Assay</th>
<th>Recovered HCG</th>
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<tbody>
<tr>
<td>American Diagnostics</td>
<td>8.9</td>
</tr>
<tr>
<td>Amersham</td>
<td>4.8</td>
</tr>
<tr>
<td>Becton Dickinson</td>
<td>7.4</td>
</tr>
<tr>
<td>Clinical Assays*</td>
<td>4.2</td>
</tr>
<tr>
<td>Corning</td>
<td>3.2</td>
</tr>
<tr>
<td>Hybritech†</td>
<td>2.8</td>
</tr>
<tr>
<td>Leeco</td>
<td>5.0</td>
</tr>
<tr>
<td>Mallinckrodt Microanalytic</td>
<td>2.7</td>
</tr>
<tr>
<td>Nuclear Medical</td>
<td></td>
</tr>
<tr>
<td>Laboratories</td>
<td>4.5</td>
</tr>
<tr>
<td>Roche Labs.</td>
<td>5.0</td>
</tr>
<tr>
<td>Serono</td>
<td>0.9</td>
</tr>
</tbody>
</table>

HCG-Pools standardized by the ZHIS 2.7–3.0 5.0–9.0 18–21 35–40 53–64 80–91

* Values obtained in actual test are twice those listed in this chart because 1st IRP is used for standardization. We used 5/9 of the value obtained to compare sensitivity and linearity with these other assays.
† Either 1st IRP or 2dIS standardization may be used.
‡ The Biopspecific Technical HCG kit gave values similar to those of Mallinckrodt's.
hCG serially diluted in male urine gives falsely low or negative values with current monoclonal hCG reagents. It is believed by us that between 30 and 75 percent of all urinary hCG are found in the form of a 12,000 D fragment. Polyclonal anti-BhCG reagents give more accurate urinary hCG values at the present time, but mixtures of monoclonal antibodies can be used to achieve similar results.

A higher molecular weight, more glycosylated hCG is sometimes found in the earliest stages of pregnancies or in certain tumor cell lines which manufacture hCG. Also, blastocysts have been shown to secrete an hCG-like molecule prior to implantation.5,8,9,15 The effect of inflammatory or enzymal degradations of hCG is unknown. Nidation, ectopic pregnancies, and pelvic inflammatory disease may alter early pregnancy forms of hCG. Future studies are needed to assess the effect of unusual molecular forms of hCG on emergency pregnancy assessment. Until that time, the use of polyclonal reagents is suggested. Rapid emergency medical protocols often utilize urinary hCG as a preliminary screen. This is followed by a quantitative B-hCG ligand assay with a true sensitivity and specificity at 3 mIU/ml to assess all negative urinary values as potentially "false".

The quantitative measurements of serum hCG shown in table II and figure I indicate that one B-hCG RIA value has an excellent chance of separating the normal from the abnormal pregnancy up to the third week postconception. This was important since in many emergency situations the physician could not wait for serial hCG-assays and an ultrasound was unable to assist in the decision-making process.12,13 Between the third and ninth week, serum hCG begins to level off, and the ability to assess gestational status may be as much as plus or minus one week. The need for quantitative serum hCG assays that assess 1 to 20 mIU per ml accurately according to the 2nd International Standard are indicated in figure 2.

A recent article has discussed the problems associated with discordant, "indeterminate", and/or false negative hCG values.11 It is suggested in figure 2 and table III that the inability of many commercial B-hCg RIA to quantitate serum hCG below 25 mIU per ml is due to lack of standardization with the 2dIS. This was noticeable even in the most linear range of these assays between 20 and 50 mIU per ml. High nonspecific binding and LH cross reactivity were also present in all hCG-RIA that could not accurately determine less than 10 mIU per ml of hCG. However, these latter defects could not approach the latitude of the error related to lack of standardization with the 2dIS. The relationship between the 1stIS, the 2dIS and the 1stIRP is confusing. Either whole hCG or its α and β subunits can be purified to homogeneity and characterized with current technology. It will become increasingly clear that the use of hCG standards in ng per ml to replace the older, less accurate biologic unitage will make sense in standardizing the quantitative immunoassay of hCG-reacting molecules in the future.

In table IV it is seen that statistically low serum BhCG assays, as defined by figure 1 and table I, were useful in accurately establishing the abnormal pregnancy with an accuracy of 80 to 100 percent, depending upon the clinical group and the week of gestation.

<table>
<thead>
<tr>
<th>Clinical Group</th>
<th>Below Normal BhCG Ranges</th>
<th>Number of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopic Pregnancies</td>
<td>13/16</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Spontaneous Abortions</td>
<td>3/3</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Incomplete Abortions</td>
<td>7/7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Threatened Pregnancies</td>
<td>3/3</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26/29</td>
<td>89.7</td>
<td></td>
</tr>
</tbody>
</table>
In contrast to hCG-RIA, ligand assay of human placental lactogen and/or pregnancy-specific B-1 glycoprotein have been used to assess late pregnancy status. Quantitative assay of hCG after the ninth week in utero is not particularly useful for evaluating the threatened conceptus. The question that remains to be answered is whether or not early gestational aging close to the time the menstrual period is missed will be useful in predicting and/or preventing postmature delivery.

In summary, these data indicate that the accurate assessment of early gestational status depends upon standardization of quantitative ligand assays with the 241S for hCG. While certain practical applications of properly standardized B-hCG-RIA have been shown, other studies may clarify unresolved peripheral questions.

References


