Comparison of 2 Commercially Available Human Chorionic Gonadotropin Immunoassays Used in the Management of Gestational Trophoblastic Neoplasia

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OBJECTIVE: To compare serum human chorionic gonadotropin (hCG) titers using 2 commercially available hCG immunoassays in patients with gestational trophoblastic neoplasia (GTN).

STUDY DESIGN: A total of 213 serum samples from 39 patients with uneventful moles and 697 serum samples from 17 patients with low-risk and high-risk GTN were obtained and subsequently measured with both the hCG C-terminal (hCG-CTP) and DPC Immulite 2000 tests.

RESULTS: In patients with uneventful moles and GTN, serum hCG levels recorded using the hCG-CTP and DPC Immulite 2000 tests correlated well (r² = 0.936 and r² = 0.958). However, the serum hCG titers measured using the DPC Immulite 2000 assay were approximately 2.5- and 2.7-fold higher than those measured with the hCG-CTP test (p < 0.0001) when lower titers of hCG (< 40 mIU/mL) were tested. In addition, 3 and 1 patient, respectively, with uneventful mole and GTN obtained positive results with the DPC Immulite 2000 test (3.0–4.2 mIU/mL) after demonstrating undetectable hCG levels with the hCG-CTP test (< 1.0 mIU/mL).

CONCLUSION: At the lower levels of hCG, a few discrepancies between hCG titers measured using the 2 commercial immunoassays arose and may be attributed to the existence of hCG-related molecules. However, these hCG metabolites disappeared rapidly, the clinical importance of which remains unclear. (J Reprod Med 2009;54:631–635)

Both commercial hCG assays, which reacted with different hCG molecules, might be useful in the management of GTN.

Keywords: DPC Immulite 2000, gestational trophoblastic neoplasms, hCG-HTP, human chorionic gonadotropin.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of 2 different subunits termed the α and β subunits. hCG is produced by trophoblast tissue in normal pregnancy, trophoblastic tumor and at low levels by poorly differentiated cancer cells. The precise determination of hCG serum levels in postmolar patients is crucial for the assessment of both the clinical course and the early detection of gestational trophoblastic neoplasia (GTN). This is especially important when low levels of hCG are measured, as inaccurate hCG titers may result in the possibility of overtreatment of a false positive result and in the discontinuation of treatment of a false negative result.1,2

Recently, several authors have reported the ex-
istence of multiple hCG-related molecules, including hyperglycosylated hCG, nicked hCG, free β-subunit, nicked free β-subunit and hCG missing β-subunit C-terminal peptide in serum samples collected from patients with GTN and that the levels of these hCG-related molecules are crucial for detecting and diagnosing GTN.3-6

In Japan, several commercially hCG assays were available for laboratory use, and the most readily available kit for managing GTN is the hCG C-terminal peptide (hCG-CTP) test (Wako Pure Chemical Industries LTD, Osaka, Japan).7 The hCG-CTP test involves the “sandwich assay” approach using antibodies raised against the hCG-CTP and hCG dimer. The hCG-CTP test detects both intact hCG and hyperglycosilated hCG. In contrast, DPC Immulite 2000 (Siemens, supplied by Mitsubishi Chemical Medience Corporation, Tokyo, Japan) detects intact hCG and multiple hCG-related molecules. Cole et al reported that appropriate hCG assays for the diagnosis and management of GTN are able to detect both intact hCG and all hCG-related molecules.8-10

The purpose of this study was to compare serial hCG titers from patients with GTN, including complete and partial mole, using the hCG-CTP test and DPC Immulite 2000 assay system.

Materials and Methods

Patients and Serum Samples

From September 2004 to December 2006, 56 patients with GTN were treated and monitored in our hospital. Of these 56 patients, 39 patients (21 patients with complete mole and 18 patients with partial mole) demonstrated spontaneous, undetectable hCG levels following weekly or biweekly hCG measurement. A total of 213 serum samples from these 39 patients with uneventful moles demonstrated spontaneous, undetectable hCG levels following weekly or biweekly hCG measurement. A total of 213 serum samples from these 39 patients with uneventful moles were subsequently measured using the hCG-CTP and DPC Immulite 2000 assay concurrently until hCG levels were undetectable via the hCG-CTP test (<1.0 mIU/mL). The remaining 17 patients were diagnosed as having high-risk GTN (5 patients) and low-risk GTN (12 patients) based on the International Federation of Obstetrics and Gynecology 2000 staging system. These patients were treated with combination and single-agent chemotherapy. A total of 697 serum samples (306 samples from patients with high-risk GTN and 391 samples from patients with low-risk GTN) were measured using the hCG-CTP and DPC Immulite 2000 assays concurrently.

Of the 306 serum samples from patients with high-risk GTN, 135 and 171 samples were obtained before and after achieving normal hCG titers using the hCG-CTP test (<1.0 mIU/mL), respectively. Similarly, 224 and 167 samples from patients with low-risk GTN were obtained before and after achieving normal hCG titers using the hCG-CTP assay.

Analysis of Serum hCG

The Wako hCG-CTP test is based on the immunometric sandwich assay approach. The labeling antibody utilized is a peroxidase conjugated polyclonal rabbit anti-hCG dimer antibody, and the second antibody is a monoclonal antibody raised against the hCG C-terminal peptide. The hCG-CTP test requires an overnight incubation period, and the measurable range of hCG without dilution is narrow (≤40 mIU/mL). The mean ± SD of serum hCG titers using the hCG-CTP test were 0.29 ± 0.37 mIU/mL in 335 (164 female and 171 male) normal Japanese volunteers, aged from 19 to 64 years old (previous multicenter study, data not shown). The upper limit of this assay was judged to be 1.0 mIU/mL (mean ± 2 SD).

The DPC Immulite 2000 assay uses 2 different monoclonal antibodies raised against the hCG β-subunit. The upper limit with this assay is reported to be 2.7 mIU/mL. The DPC Immulite 2000 assay labels all hCG-related molecules, and the use of this assay system is recommended in the diagnosis and monitoring of patients with GTN.

Results

Serum hCG Titers of Patients with Uneventful Moles

The serum hCG titers measured using hCG-CTP and DPC Immulite 2000 tests correlated well (Y = 1.359X-1128.23; r² = 0.936, N = 213). Moreover, in 129 serum samples, a high correlation of low level hCG (<40 mIU/mL) was observed (Figure 1). Although the hCG titers correlated well (r² = 0.941) using these 2 assay systems, the serum hCG titers measured using the DPC Immulite 2000 assay were approximately 2.5-fold higher than those measured using the hCG-CTP test.

In addition, when serum hCG levels were undetectable (<1.0 mIU/mL) using the hCG-CTP test, mean hCG titers recorded with the DPC Immulite 2000 test were on average 1.5 mIU/mL (ranged from 1.0 to 4.2 mIU/mL). This included 3 patients (7.7%) who recorded positive results (3.0–4.2 mIU/mL) (Table I). These 3 patients subsequently
were followed using the hCG-CTP assay alone. During the observational period (23–210 weeks), 38 patients demonstrated no evidence of disease; however, 1 patient developed high-risk GTN 27 months following evacuation of a mole. In addition, the mean serum hCG titers measured with the DPC Immulite 2000 assay were significantly higher at 2–4 weeks before achieving undetectable hCG levels in comparison to those measured with the hCG-CTP assay (p<0.0001). Serum hCG Titers of Patients with Low-Risk and High-Risk GTN

Of the 697 samples measured, 359 were obtained prior to achieving normal hCG titers using the hCG-CTP test. The correlation between the 2 hCG assay systems for these samples was high (Y = 1.1×X+551.3, r² = 0.958), and the coefficient of correlation was similar to that observed for patients with uneventful moles. In addition, the correlation of low levels of hCG (<40 mIU/mL) was compared in 232 serum samples (Figure 2). The correlation was high (r² = 0.909), and each serum hCG titer observed using the DPC Immulite 2000 assay was approximately 2.7-fold higher than those measured using the hCG-CTP assay.

When the serum hCG levels were undetectable using the hCG-CTP test (n=17), median hCG titers measured by the DPC Immulite 2000 assay were approximately 1.0 mIU/mL (range, 1.0–3.0 mIU/mL). Only 1 patient with high-risk GTN obtained a positive result (3.0 mIU/mL) with the DPC Immulite 2000 assay. However, 4 days later, the hCG titer dropped to 1.4 mIU/mL and remained at an undetectable level thereafter.

After recording undetectable levels using the hCG-CTP assay (<1 mIU/mL), 338 serum samples

Table 1: hCG Regression Pattern in Uneventful Postmolar Patients with the hCG-CTP Test and DPC Immulite 2000 Assay

<table>
<thead>
<tr>
<th>Weeks before achieving undetectable hCG levels with the HCG-CTP test</th>
<th>Case</th>
<th>CTP</th>
<th>DPC</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>39</td>
<td>0.6±0.2</td>
<td>1.5±0.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>2.9±1.2</td>
<td>6.4±4.4</td>
<td>0.0186</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>5.4±6.7</td>
<td>12.8±17.7</td>
<td>0.0108</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>10.4±11.2</td>
<td>26.8±33.1</td>
<td>0.0127</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>85.9±259.9</td>
<td>127.6±354.6</td>
<td>0.067</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>2,900.1±11,806.3</td>
<td>4,377.1±18,051.4</td>
<td>0.316</td>
</tr>
</tbody>
</table>
collected from 17 patients were also measured using both hCG assays. Four samples (1.2%) from 3 patients demonstrated transient, high hCG titers (3.0–14.4 mIU/mL) with the DPC Immulite 2000 (>2.7 mIU/mL) test. All of these 17 patients could possibly have been in remission following chemotherapy treatment ranging from 13 to 30 months.

Discussion
The reliability of commercially available assays in the measurement of hCG serum levels has recently proven to be problematic. This is thought to be due in part to the reported existence of heterophilic antibodies, which may cause false positive results and needless treatments in some patients.\(^1,11\) Moreover, variation in hCG levels measured using different assay systems creates a problem in the monitoring of patients with GTN and cancer, as false positive results may result in overtreatment, while false negative levels may result in the premature discontinuation of treatment and a subsequent increase in the risk of disease relapse.\(^10\) In patients with GTN, multiple forms of hCG, including intact hCG, hyperglycosylated hCG, nicked hCG, free β-subunit, nicked free β-subunit and hCG molecules missing the C-terminal peptide of the β-subunit, are known to exist in serum.\(^3,6\) These hCG molecules are not thought to be secreted by trophoblast cells but rather are thought to arise following nicking and dissociation from intact and hyperglycosylated hCG by elastases produced by macrophages and neutrophils associated with trophoblastic cells.\(^3,12,13\) Cole et al\(^10\) reported that the DPC Immulite 2000 assay and other similarly designed hCG tests were able to detect all forms of hCG molecules and that these assays may be critical for the appropriate management of GTN.

In the current study, we compared the correlation and decay of serum hCG titers in patients with GTN as measured using the hCG-CTP and DPC Immulite 2000 tests. In cases with uneventful moles, the overall correlation of serum hCG titers measured using the hCG-CTP and DPC Immulite 2000 assays was high (\(Y = 1.359 \times X - 1128.23; r^2 = 0.936\)). However, in patients with low levels of hCG titers (<40 mIU/mL), the serum hCG titers measured via the DPC Immulite 2000 test were approximately 2.5-fold higher than those obtained using the HCG-CTP test. Similar results were also observed in patients with low- and high-risk GTN. Although we could not measure the levels of hCG-related molecules separately, the discrepancies observed for the low level hCG titers between the 2 commercially available kits may be attributed to differences in the recognition of hCG and its related molecules.\(^5\)

In contrast, the decay of serial hCG titers following the evacuation of mole and during chemotherapy was similar for both the hCG-CTP and DPC Immulite 2000 assays. The time required to achieve levels within the normal range with the 2 kits was similar in 92% of uneventful molar patients (36 of 39) and 95% of patients with low- and high-risk GTN (18 of 19). In the additional 4 patients, abnormal hCG titers recorded with DPC Immulite 2000 subsequently decreased rapidly to within the normal range.

In conclusion, we compared serum hCG titers measured using the hCG-CTP test and DPC Immulite 2000 in patients with GTN, including those with uneventful moles. From this small study, both commercial hCG assays, which reacted with different hCG molecules, might be useful in the management of GTN. The significant differences in serum hCG titers measured using these 2 kits suggested the presence of hCG-related molecules in the serum of patients with GTN. In addition, we demonstrated that an almost equal amount of time is required to attain undetectable hCG levels using these 2 kits, suggesting that the hCG-related molecules degrade and disappear rapidly.

References


