The Clinical Evaluation of the Simultaneous Measurements of Human Chorionic Gonadotropin (hCG) and its Alpha-subunit in Sera of Patients with Trophoblastic Diseases*

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Synopsis

The concentrations of human chorionic gonadotropin (hCG) and its free immunoreactive alpha-subunit (hCG-α) in the sera of patients with trophoblastic diseases were measured by hCG and hCG-α radioimmunoassay (RIA), respectively.

In the sera of 12 women with hydatidiform mole large amounts of hCG and considerably high level of hCG-α were detected in all cases. After the evacuation of mole the serum level of these glycoproteins decreased, the level of hCG-α declined more rapidly than hCG.

In the sera of patients with destructive mole the concentration of hCG-α was usually lower than that of hCG. After hysterectomy and chemotherapy the levels of hCG-α declined practically paralleling that of hCG. However, when hCG had decreased to undetectable level, hCG-α could no longer be detected in all cases.

Although in the serum of patient with choriocarcinoma involving the uterus and lungs the concentration of hCG-α was almost as high as that of hCG, the secretory pattern of hCG and hCG-α might not be closely related. The changes in the serum level of free hCG-α as well as that of hCG paralleled the clinical course of the patients examined in this study. The present results suggest that measurements of the serum free hCG-α may be a useful parameter to follow the clinical course and to evaluate the efficacy of treatments of trophoblastic diseases.

Measurements of human chorionic gonadotropin (hCG) in urine and serum have been established as useful clinical parameter for the management of trophoblastic diseases. hCG is a glycoprotein hormone composed of two non-identical alpha- and beta-subunit (Swaninathan and Bahl, 1970). The α-subunit is common among the human glycoprotein hormones while the α-subunit is hormone specific (Pierce et al., 1971). By utilizing the antiserum against the β-subunit of specific glycoprotein hormone, it can be measured even in the presence of other glycoprotein hormones. It is especially clinically useful to measure specifically low titer of hCG in the serum of patients with trophoblastic diseases in the presence of LH by using the antibody against hCG-β (Vaitukaitis et al., 1972).

The production and secretion of free subunits have been also demonstrated physiological conditions (Franchimont and Reuter, 1972) and neoplastic diseases (Weintraub and Rosen, 1973). We previously demonstrated that hCG and its free subunits are present in the serum and chorionic

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tissue of normal as well as in molar pregnancy (Ashitaka et al., 1974, 1974, 1977).

The present studies were undertaken to measure the concentration of hCG-α in the sera of patients with trophoblastic diseases undergoing surgical and chemical treatments. Comparative studies of the levels of hCG-α with those of hCG in the same samples were carried out to evaluate the clinical usefulness of measuring serum hCG-α as a follow-up test of patient with trophoblastic diseases.

**Materials and Methods**

**Blood samples**

Samples of blood were obtained from 12 patients with hydatidiform mole which was confirmed by the evacuation of molar vesicles, 6 patients with destructive mole and 2 patients with choriocarcinoma. The blood allowed to be clotted was centrifuged and the serum was separated and stored at -20°C until assayed.

**Radioimmunoassay methods**

The concentrations of hCG were measured simultaneously both by homologous hCG radioimmunoassay (RIA) and hCG-α RIA. The concentrations of hCG-α were measured by homologous hCG-α RIA. Highly purified hCG (CR-119) and hCG-Kobe (Ashitaka et al., 1970) were served as reference preparations for hCG assay. The sub-units of hCG were prepared by the urea treatment as described by Swaminathan and Bahl (1970). hCG and its sub-units (Kobe) were labeled by an enzymatic iodination method with Na125I to specific activities of hCG: 60-80 μCi/μg, hCG-α: 72-88 μCi/μg, hCG-β: 56-62 μCi/μg (Ashitaka and Koide, 1974).

Radioimmunoassay results were calculated from the linear regression curve (Ordinate: logit scale expressed as a percentage of B/T, Abscissa: log scale of antigen concentration) as described by Rodbard (1974).

As hCG and its subunits were cross-reacted with each other in the respective homologous RIA system, actual amounts of these glycoproteins in the samples were calculated from the formulas described in a previous report (Ashitaka et al., 1974).

**Case report**

3 patients, 29-36 years old women (case A, B, C), had cervical dilatation and evacuation for hydatidiform mole. At 20-30 days after the evacuation procedure the levels of serum hCG stopped declining and instead started to increase gradually. From the findings of their pelvic angiography (PAG) the recurrence of trophoblastic diseases was suspected, and abdominal hysterectomy was performed. The pathologic diagnosis of the excised uterine tissue was destructive mole. The chest X-ray revealed metastatic tumor shadows in the lung of all three cases.

Case D, 24 year-old woman underwent evacuation for hydatidiform mole. The chest X-ray revealed a coin lesion already in molar pregnancy, she was treated with 3 courses of methotrexate (MTX) immediately after the removal of the mole. On the 60th day after curettage the serum hCG was no longer detectable and the coin lesion had disappeared in the chest X-ray.

Case E, 29 year-old woman had evacuation for hydatidiform mole in October, 1975. She had been not received any therapy till March, 1978. At this time the concentration of serum hCG increased gradually and a routine chest X-ray revealed metastatic lesions of the lung. The PAG revealed no abnormal findings. Following one course of Actinomycin-D (Act-D) the concentration of hCG in the serum decreased gradually and was at the undetectable level after two additional courses of Act-D.

Case F, 31 year-old woman, gravida 5, para 1, molar pregnancy 2, underwent evacuation in November, 1977, for a hydatidiform mole. She was asymptomatic until February, 1978, when the serum concentrations of hCG increased gradually. No metastatic lesion was found in the chest X-ray and no abnormal findings were detected by PAG. Even after a course of MTX the serum value of hCG continued to increase until she underwent abdominal hysterectomy. The pathologic diagnosis was choriocarcinoma. For about 30 days after the surgery she was given two courses of MTX and serum hCG fell to the undetectable level.

Case G, 26 year-old woman, was found to have a molar pregnancy in February, 1974. Twenty days after evacuation she had metastatic lesions of the right lung demonstrated by the chest X-ray, underwent hysterectomy and was given MTX and Act-D post-operatively. Thereafter, she had done well until August, 1975, when a routine chest X-ray revealed metastatic lesions of the right lung again and the concentration of the serum hCG increased gradually. Five courses of Act-D were given and the serum hCG became undetectable by hCG-RIA and all the metastatic lesions except one cleared in chest X-ray. In October, 1975, the segmental and wedge resection of the right lung were performed for the remaining metastases. The pathologic diagnosis of the lung was ascertained to be the metastases of choriocarcinoma. From the 30th day after operation of the lung, two courses of Act-D were given. After that, the chest X-ray was clear, and the concentration of serum hCG was no longer detectable. In April,
1977, the concentration of serum hCG increased gradually and the chest X-ray revealed two metastatic lesions in the right lung. After three courses of Act-D the serum hCG became undetectable and the chest X-ray only showed a small residual shadow of metastasis in the right lung. She had not received any therapy during the routine follow-up until an enlargement of the shadow was found in the chest X-ray in January, 1978. Then she was given 170 mg of MK 631 (derivatives of bleomycin) for a month. In March, 1978, she underwent wedge resection of the right lung and the three tumorous lesions were resected. Following three courses of Act-D, the concentration of hCG decreased and the chest X-ray showed no metastatic lesion. Presently she was under strict follow-up using routine hCG titration and chest X-ray.

Results

Radioimmunoassays of hCG and its sub-units

The standard lines for RIA of hCG, hCG-α and hCG-β are shown in Fig. 1-a, b, c. The accuracies of each assay were described in the previous report (Ashitaka et al., 1977). The sensitivity of hCG RIA was 0.8 ng/ml and a 50% intercept was 6.5 ng/ml. The sensitivity of hCG-α RIA was 0.5 ng/ml and a 50% intercept was 7.0 ng/ml. The sensitivity of hCG-β was 0.2 ng/ml and a 50% intercept was 1.8 ng/ml.

The concentrations of hCG and hCG-α in the sera of women with hydatidiform mole

The concentrations of hCG in the sera of women with hydatidiform mole ranged 15 to 210 μg/ml with a mean level of 84.2 μg/ml (±SE: 15.8). This value was significantly higher than that in sera of normal pregnant women of the corresponding gestational weeks. The concentration of hCG did not correlate with the sequelae of the patient after removal of mole (Table 1).

The concentration of hCG-α in the sera of women with hydatidiform mole ranged from 220 to 2,050 ng/ml and the mean level was 1,067 ng/ml (±SE: 137). There was no significant correlation between the concentration of hCG and that of hCG-α. The concentration of hCG-α did not definitively reflect the clinical course of the patient after removal of mole. In all cases, the sera levels of hCG-α were significantly lower than those of hCG (Table 1).

The concentrations of hCG and hCG-α in the sera of patients with the sequential changes of trophoblastic diseases

The gynecological histories of the patients with the sequential changes of trophoblastic diseases are shown in Table 2. All the antecedent pregnancies were the hydatidiform mole. One patient (case F) had experienced two molar pregnancies. All the cases received chemotherapy and underwent abdominal hysterectomy after the removal of the mole except two patients (case D and E).

Table 1. Serum concentrations of hCG and hCG-α measured by the respective RIA in 12 patients with hydatidiform mole.

<table>
<thead>
<tr>
<th>Case</th>
<th>Gestation (weeks)</th>
<th>hCG (ng/ml)</th>
<th>hCG-α (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>15,000</td>
<td>1,300</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>34,000</td>
<td>1,550</td>
</tr>
<tr>
<td>3*</td>
<td>13</td>
<td>97,650</td>
<td>640</td>
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<tr>
<td>4</td>
<td>14</td>
<td>145,000</td>
<td>1,050</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>55,000</td>
<td>310</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
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</tr>
<tr>
<td>7*</td>
<td>12</td>
<td>210,000</td>
<td>1,650</td>
</tr>
<tr>
<td>8*</td>
<td>14</td>
<td>32,000</td>
<td>290</td>
</tr>
<tr>
<td>9</td>
<td>16</td>
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</tr>
<tr>
<td>11*</td>
<td>13</td>
<td>48,700</td>
<td>1,540</td>
</tr>
<tr>
<td>12</td>
<td>17</td>
<td>113,000</td>
<td>2,050</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>84,238</td>
<td>1,067</td>
</tr>
<tr>
<td>± SE</td>
<td></td>
<td>15,827</td>
<td>173</td>
</tr>
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</table>

* The cases underwent abdominal hysterectomy after removal of mole and pathologic diagnosis was destructive mole.
Fig. 1. Dose-response lines for hCG (CR 119), hCG-α, hCG-β, hLH (LER 960) and hFSH (LER 1366) in each homologous system -a: hCG RIA, -b: hCG-α RIA, -c: hCG-β RIA
Table 2. The cases with the sequential changes of trophoblastic disease after removal of mole.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Gravida</th>
<th>Para</th>
<th>Molar Pregnancy</th>
<th>Abdominal Hysterectomy</th>
<th>Pathologic Diagnosis</th>
<th>Metastasis</th>
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<tbody>
<tr>
<td>(A)</td>
<td>30</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>(+)</td>
<td>Destructive Mole</td>
<td>lung</td>
</tr>
<tr>
<td>(B)</td>
<td>36</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>(+)</td>
<td>Destructive Mole</td>
<td>lung</td>
</tr>
<tr>
<td>(C)</td>
<td>79</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>(+)</td>
<td>Destructive Mole</td>
<td>lung</td>
</tr>
<tr>
<td>(D)</td>
<td>24</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>(-)</td>
<td>Undetermined</td>
<td>lung</td>
</tr>
<tr>
<td>(E)</td>
<td>29</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>(-)</td>
<td>Undetermined</td>
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<td>(F)</td>
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<td>5</td>
<td>1</td>
<td>2</td>
<td>(+)</td>
<td>Choriocarcinoma</td>
<td>(-)</td>
</tr>
<tr>
<td>(G)</td>
<td>26</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>(+)</td>
<td>Choriocarcinoma</td>
<td>lung</td>
</tr>
</tbody>
</table>

After the removal of mole the concentration of serum hCG-α decreased more rapidly than that of hCG in all cases (case A, B, C and D in Fig. 2, 3, 4 and 5). In the sera of patients undergoing chemotherapy and surgical treatments the secretory pattern of hCG-α practically paralleled that of hCG in all cases.

In the sera of patients with destructive mole the concentration of hCG-α was usually lower than that of hCG. Following hysterectomy and chemotherapy the levels of hCG-α declined gradually almost in parallel with those of hCG, but when hCG became undetectable hCG-α was already no longer detected in all cases (case A, B, C, D and E in Fig. 2, 3, 4, 5 and 6).

Fig. 2. Clinical course of the patient (case A) with destructive mole involving the uterus and lung, showing serum hCG and hCG-α

Act-D = Actinomycin-D

Fig. 3. Clinical course of the patient (case B) with destructive mole involving the uterus and lungs, showing serum hCG and hCG-α

MTX = Methotrexate

Fig. 4. Clinical course of the patient (case C) who had already the pulmonary metastases before the evacuation of mole, showing serum levels of hCG and hCG-α
Fig. 5. Clinical course of the patient (case D) with trophoblastic disease classified into undetermined group because of not undergoing the surgery, showing serum levels of hCG and hCG-α.

Fig. 6. Clinical course of the patient (case E) with trophoblastic disease classified into undetermined group because of not undergoing the surgery, showing serum levels of hCG and hCG-α.

Fig. 7. Clinical course of the patient (case F) with choriocarcinoma having no pulmonary metastasis, showing serum levels of hCG and hCG-α.

Fig. 8. Clinical course of the patient (case G) with choriocarcinoma involving the uterus and lungs, showing serum hCG and hCG-α.
Case D showed the greatest discrepancy in the concentrations of hCG and hCG-α. Even when the concentration of hCG was over 1,000 ng/ml and showed a tendency to rise, the concentration of hCG-α declined rapidly and did not show any significant fluctuation. After three courses of MTX when hCG became undetectable, hCG-α had already reached the undetectable level about twenty day before (Fig. 5).

In case F, a patient with choriocarcinoma, after one course of MTX the concentration of serum hCG continued to increase while that of hCG-α started to fall by this time. After hysterectomy the levels of both hCG and hCG-α declined rapidly (Fig. 7).

In case G, she underwent wedge resections of the right lung but the concentrations of serum hCG and hCG-α did not decline immediately. hCG and hCG-α became undetectable after chemotherapy. The secretory pattern between hCG and hCG-α might not be closely related (Fig. 8).

Discussion

It has been well known that there is a tendency for hCG to be secreted in large quantities during the molar pregnancy than in normal pregnancies. In the present studies it has been confirmed that large quantities of hCG are presented in the sera of patients with hydatidiform mole as compared with normal pregnant women.

Furthermore, relatively high amounts of hCG-α were detected in the sera of patients with hydatidiform mole. As previously described (Ashitaka et al., 1977), the fluid of molar vesicles obtained at the time of evacuation contained higher concentration of hCG-α than the sera of matched patients.

After the evacuation of the mole the serum level of hCG-α declined rapidly almost in parallel with that of hCG. These results suggested that hCG-α produced by the trophoblastic tissue was secreted into the circulation independently of hCG in molar pregnancy as shown in normal pregnancy.

In the sera of all the cases with hydatidiform mole examined in this studies hCG-α declined more rapidly than hCG after the removal of mole. This can be attributed to the differences in the plasma half life between hCG-α and hCG (Braunstein et al., 1972). We previously demonstrated that the secretion of hCG-α by the term placenta exceeded that of hCG in experiments using the in vitro organ perfusion system. hCG-α should be secreted by the trophoblastic tissue at a higher concentration than that detected in peripheral circulation because of the short half life of hCG-α.

The relationship between the secretory pattern of the serum hCG-α and the outcome were investigated. In case A, destructive mole, for about ten days after the evacuation of the mole hCG and hCG-α declined rapidly, and for the subsequent twenty days the hCG-α level increased, on the other hand, hCG level decreased gradually. This case had pulmonary metastases and was given chemotherapy post-operatively and after two courses of Act-D the serum hCG was no longer detectable. At this time the serum hCG-α had already reached the undetectable level about ten days earlier.

It is interesting to note that in case D, E, F which responded satisfactorily to chemotherapy the serum hCG-α had reached the undetectable level about ten days earlier than the serum hCG. On the other hand, in cases B, C and G which were resistant to chemotherapy the serum hCG-α could be still more detected almost until the serum hCG became undetectable. In these cases, especially with case G, the concentration of serum hCG-α was almost as high as that of hCG and the serial secretory patterns did not parallel each other while undergoing chemotherapy.
Vaitukaitis and Ebersole (1975) showed the presence of hCG and its subunits in the extracts of tumor tissue, serum and urine from women with trophoblastic diseases and also found that intact hCG but no free subunit existed in the sera of patients which responded chemotherapy. We had showed previously that free hCG-α existed in the serum in molar pregnancy assayed directly by RIA.

Secretion of free subunits of glycoprotein hormones by the pituitary was demonstrated by many investigators (Prentice and Ryan, 1975; Kourides et al., 1975; Kaplan et al., 1976). Bilateral oophorectomy may induce a hypergonadotropic status and free α-subunit may be secreted in higher concentration by the pituitary than suspected. In molar pregnancy, however, pituitary function is suppressed as in normal pregnancy (Nakano et al., 1975). Edmonds et al. (1975) showed that α-subunit of hLH was secreted by the anterior pituitary in response to LRF. But the peak concentration of it was less than 3 ng/ml. Hence, it is inconceivable that such large quantities of free α-subunit as demonstrated in this study are secreted by the pituitary in molar pregnancy.

Dawood et al. (1977) demonstrated that in patients with choriocarcinoma who eventually developed cerebral metastases, hCG-α increased while hCG and hCG-β declined or were absent. They speculated that the persistence of the disease might be missed, should the serum hCG by RIA with hCG-β subunit antiserum be negative, because the small focus of tumor cells might be producing predominantly free α-subunit and fragment of hCG which were not antigenic to the hCG-β antibody. As they fail to examine the declining pattern of serum hCG-α until the level fell within the sensitivity range of the assay, the correlation between the changes in serum hCG-α levels and the clinical courses of the patients is not clear.

In patients with trophoblastic disease and also with non-trophoblastic neoplasia the secretion of hCG and its free subunits have been demonstrated (Weintraub et al. 1973). Tashjian et al. (1973) demonstrated that the clones of ectopic hormone-producing cells from a bronchogenic carcinoma showed different rates of synthesis and secretion of hCG and its α and β subunit, the amount of one or the other subunits always exceeded that of the complete hormone molecule. Lieblich et al. (1976) found that HeLa strains derived from a carcinoma of the cervix secreted α-subunit and suggested that the α-subunit might turn out to be relatively easily derepressed and be a more primitive protein. Ghosh and Cox (1976) showed that HeLa cells secreted hCG-α and speculated the putative derepression of an element of the genome with the synthesis of a protein normally produced only by the specialized trophoblastic cells. Landefeld et al. (1976) and Daniels-McQueen et al. (1978) showed that using mRNA prepared from placenta α and β subunit of hCG were synthesized individually in a wheat germ cell free system. It is probable that the synthesis of hCG is performed through the subunits in the trophoblastic tissue. These data suggest that some genetic changes may happen in the process of hCG synthesis by the trophoblast in the course of sequential malignant changes and consequently, the unbalanced secretion of hCG and its subunits may occur.

It is already recognized that the measurements of hCG by hCG-β RIA using anti hCG-β serum are reliable clinical parameter in the follow-up study of the patients with trophoblastic diseases. In the present study the serial levels of hCG by hCG-β RIA correlated well with the clinical course of the patients undergoing chemotherapy and surgical treatments.

In addition to hCG, the levels of hCG-α paralleled the clinical course of the patients
examined in this study. Moreover, the response to therapies was more rapidly reflected by the changes in the levels of hCG-α than by hCG.

Based on the present study measurements of the serum free hCG-α may be a useful parameter to detect the sequential changes and to evaluate the efficacy of treatment in the management of trophoblastic diseases.

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References


