Elevation of Free β Subunit of Human Choriogonadotropin and Core β Fragment of Human Choriogonadotropin in the Serum and Urine of Patients with Malignant Pancreatic and Biliary Disease

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ABSTRACT

Human choriogonadotropin (hCG), its free β subunit (βhCG), and the core β fragment (cβhCG) were measured by highly sensitive time-resolved immunofluorometric assays in the serum and urine of 29 patients with pancreatic cancer, 7 patients with biliary cancer, and 45 patients with benign pancreatic or biliary diseases. The results were compared with those of an age- and sex-matched reference population of nonpregnant women and men. Of the various forms of hCG assayed in serum, βhCG showed the best diagnostic accuracy, and cβhCG was the best marker in urine. Elevated serum concentrations of βhCG were observed in 72% of the patients with pancreatic cancer, in 6 of 7 patients with biliary cancer, and in 9% of those with benign disorders. The serum concentrations of cβhCG were elevated in 45%, 57%, and 2%, respectively, and those in urine in 55%, 71%, and 11%, respectively. The molar concentrations of cβhCG in serum were mostly lower than those of βhCG. Thus βhCG secreted into serum appears to be the main source of cβhCG in urine. Provided that they are measured by sufficiently sensitive and specific assays, βhCG in serum and cβhCG in urine appear to be useful markers for pancreatic and biliary cancer.

INTRODUCTION

hCG is a glycoprotein hormone consisting of two dissimilar subunits designated α and β. In serum and urine, hCG immunoreactivity occurs in many different molecular forms. During pregnancy intact hCG is the main form in serum (1). The proportion of βhCG is 1–8% (2–6), and that of the so-called core fragment of βhCG comprises only about 0.03% of total hCG immunoreactivity (7, 8). In urine cβhCG is the major form of hCG (9, 10). In nonpregnant women and men the concentrations of hCG in serum (11) and hCG, βhCG, and cβhCG in urine increase with age. The serum concentrations of βhCG are similar in women and men and they change very little with age, while in women they are 10–20% of those of hCG.

hCG is an established marker for trophoblastic diseases, in which the level of βhCG in serum may also be elevated (12). In trophoblastic cancer the ratio of βhCG to hCG is higher than in benign molar disease or pregnancy (13–15). Elevated levels of hCG and βhCG are frequently observed in nonseminomatous testicular cancer (16), and in 1–2% of the patients, βhCG is the only form of hCG found in serum (17). Thus, hCG and βhCG are important serum markers for these cancers.

Elevated serum levels of hCG immunoreactivity are occasionally seen in patients with nontrophoblastic neoplasms like cancer of the stomach (23%), liver (17%), lung (19–33%), and urinary bladder (30%) (18–20), as well as adenocarcinoma (33–50%) (18) and endocrine carcinoma of the pancreas (69%) (21, 22). Elevated levels of serum hCG have also been observed in 10–18% of patients with nontrophoblastic gynecological cancer, but the hCG level in serum does not correlate with the course of the disease, especially when treatment has included ovariectomy. Therefore hCG in serum has not been considered a useful marker for these cancers (23, 24).

When sensitive and specific methods are used to measure hCG, concentrations in the serum of fertile women and men are below 2 IU/liter, but they increase after the menopause to 2–9 IU/liter (11). This may explain some earlier observations of elevated serum levels in cancer patients. When postmenopausal levels were considered Ozturk et al. (4) did not find elevated serum levels (>1.5 μg/liter) of intact hCG in any of 178 patients with various types of cancer.

In contrast to hCG in serum, hCG immunoreactivity in urine appears to be a useful test for cancer. Papapetrou et al. (25) observed elevated levels in 44% of patients with various nontrophoblastic malignancies, whereas the hCG levels in serum were elevated in only 17%. The main immunoreactivity in urine was shown to be a fragment of hCG (26). Later studies have confirmed that cβhCG is often elevated in the urine of patients with gynecological cancer (27, 28), and the levels correlate with the progress of the disease (29). Some patients with highly elevated levels of cβhCG in urine have βhCG in serum (25, 30), suggesting that cβhCG in urine is derived from βhCG.

To evaluate the usefulness of various hCG assays for the diagnosis of pancreatic cancer and to clarify the origin of cβhCG in the urine of cancer patients, we have determined serum and urine levels of hCG, βhCG, and cβhCG by highly sensitive assays. To evaluate the cancer specificity of hCG immunoreactivity we also studied patients with benign disease.

MATERIALS AND METHODS

Patient Samples. Parallel samples of serum and urine were obtained from patients with ductal adenocarcinoma of the pancreas (n = 29), pancreatitis (n = 25), biliary cancer (n = 7), and benign biliary obstruction (n = 20) (Table 1). Four patients with pancreatic cancer had resectable tumors. All samples were stored at −20°C until analyzed.

Assays. hCG, βhCG, and cβhCG were quantitated in serum and urine by IFMAs as previously described (5, 7, 11). The sensitivity of the assays for hCG, βhCG, and cβhCG was 0.8, 0.45, and 0.44 pmol/liter, respectively. Cross-reaction of hCG and βhCG in the assay for cβhCG was 1.2% and 37%, respectively. Cross-reaction of hCG and cβhCG in the assay for βhCG was 0.15% and <0.1%, respectively. In the hCG assay, the cross-reactions of βhCG and cβhCG were 0.6% and <0.1%, respectively.

Received 12/31/91; accepted 6/23/92.

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1 Supported by grants from the Finnish Cancer Foundation, the Finnish Social Security Institution, the Sigrid Juselius Foundation, and the Magnus Ehrnrooth Foundation. Parts of the results have been presented as an abstract at the Fifth World Congress on Gestational Trophoblastic Diseases, London, October 3–5, 1990.

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3 The abbreviations used are: hCG, human choriogonadotropin; βhCG, free β subunit of hCG; cβhCG, core β fragment; IFMA, time-resolved immunofluorometric assay; ROC, receiver operating characteristic.

**Table 1** Age distribution of the patients studied

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic cancer</td>
<td>29</td>
<td>73</td>
<td>45–91</td>
</tr>
<tr>
<td>Biliary cancer</td>
<td>7</td>
<td>78</td>
<td>30–81</td>
</tr>
<tr>
<td>Benign diseases</td>
<td>45</td>
<td>56</td>
<td>18–91</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>14</td>
<td>36</td>
<td>18–66</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>11</td>
<td>42</td>
<td>24–71</td>
</tr>
<tr>
<td>Benign biliary obstruction</td>
<td>20</td>
<td>73</td>
<td>51–91</td>
</tr>
</tbody>
</table>

respectively. The results for cβhCG in serum were corrected for cross-reaction with βhCG.

Creatinine in urine was quantitated by the modified Jaffé method (31) with a Kone Progress analyzer (Kone Instruments, Espoo, Finland), using reagents supplied by the manufacturer.

Gel Chromatography. One hundred-μl samples of serum (n = 3) and 1-ml samples of urine (n = 4) were fractionated by gel chromatography on a 1.6 x 40 cm column of Sephadex G-75 (Pharmacia Fine Chemicals, Uppsala, Sweden). Elution was performed with 50 mmol/liter Tris (pH 7.7), 0.15 mol/liter NaCl, and 0.05 g/liter NaN₃. The flow rate was 15 ml/h, and 1-ml fractions were collected into tubes prefilled with 100 μl assay buffer with a 10-fold protein concentration. hCG, βhCG, and cβhCG were quantitated in the fractions by IFMAs using a sample volume of 200 μl. The elution profiles of the samples were compared with those of purified preparations of urinary hCG, βhCG (5), and cβhCG (32).

Statistical Analysis. The correlation between measurable concentrations of different markers was analyzed by the Spearman test. ROC curve analysis was performed as described by Metz (33).

Reference Intervals. The reference intervals for hCG, βhCG, and cβhCG in serum and urine have been established in nonpregnant women and men. The upper reference limit of hCG in the serum of women below and above 50 years of age is 8.6 and 15.5 pmol/liter, respectively. The upper reference limit of cβhCG in serum and urine was 1.1 pmol/liter. The upper reference limit of βhCG in serum was based on the 97.5 percentile of the reference group matched for age and sex.

<table>
<thead>
<tr>
<th>Cutoff levels based on the 97.5 percentile of a reference group matched for age and sex.</th>
<th>n</th>
<th>hCG</th>
<th>βhCG</th>
<th>cβhCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic cancer</td>
<td>29</td>
<td>4 (14%)</td>
<td>21 (72%)</td>
<td>13 (45%)</td>
</tr>
<tr>
<td>Biliary cancer</td>
<td>7</td>
<td>1 (14%)</td>
<td>6 (86%)</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>Benign diseases</td>
<td>45</td>
<td>3 (7%)</td>
<td>9 (9%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>14</td>
<td>0 (0%)</td>
<td>1 (7%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>11</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Benign biliary obstruction</td>
<td>20</td>
<td>3 (15%)</td>
<td>3 (15%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Conversion Factors. International units of hCG (third International Standard) were converted to molar units by using an Mᵣ of 36,700 and a specific activity of 9,286 IU/mg (34). For βhCG an Mᵣ of 22,000 was used (35), and for cβhCG an Mᵣ of 10,000 (32).

**RESULTS**

hCG, βhCG, and cβhCG in Serum. Elevated concentrations of βhCG in serum were observed in 72% of the patients with pancreatic cancer and in 9% of those with benign diseases (Fig. 1; Table 2). In pancreatic cancer the median concentration was 2.4-fold and the highest level 120-fold the cutoff level. In patients with benign diseases the median level was 39% of the cutoff level and the maximum elevation 2.2-fold. In biliary cancer βhCG was elevated in 6 of 7 patients (86%) (Fig. 1). hCG in serum was elevated in 4 (14%) patients with pancreatic cancer, and the highest level was 1.8-fold the cutoff level. cβhCG was elevated in 45% of the patients with pancreatic cancer and in 4 of the 7 (57%) patients with biliary cancer. The highest value was 86-fold the cutoff level. The concentrations of βhCG were higher than those of cβhCG in all but three samples. In these samples the elevation was small, 1.1–2.3-fold, and the concentration of βhCG was normal. Of the six patients with normal serum levels of βhCG two had a small resectable tumor.

hCG, βhCG, and cβhCG in Urine. Elevated concentrations of cβhCG in urine were observed in 55% of the patients with pancreatic cancer and in 11% of those with benign disease. The corresponding figures for urinary βhCG were 28% and 13%, respectively (Fig. 2; Table 3). The highest elevation of urinary cβhCG in pancreatic cancer was 58-fold, and in benign disease it was 1.7-fold the cutoff level. In biliary cancer urinary βhCG
was elevated in 4 of 7 (57%) and cßhCG was elevated in 5 of 7 patients (71%) (Fig. 2). The hCG levels in urine were normal in all samples.

Follow-up of Patients with Pancreatic Cancer. In a patient with a resectable pancreatic tumor the concentrations of βhCG in serum decreased after surgery and started to rise 2 months before a recurrence was clinically observed (Fig. 3). In another two patients with normal preoperative serum levels of βhCG no elevation was seen in connection with a recurrence (not shown).

Receiver Operating Characteristics. ROC curve analysis was used to evaluate the diagnostic value of various forms of hCG and to determine the optimal cutoff level for differentiation between pancreatic cancer and benign pancreatic and biliary disease. This analysis showed that βhCG in serum was a better marker than cßhCG in urine (Fig. 4). The other forms of hCG in serum and urine had much lower sensitivity and specificity. The cutoff value giving 90% specificity in benign disease was 1.7 pmol/liter for serum βhCG and 1.02 pmol/mmol creatinine for urinary cßhCG. Using these cutoff levels the frequency of elevated levels of βhCG in the serum of patients with pancreatic cancer was 72%, and that of urinary cßhCG was 59%.

Correlations between the Concentrations of Different Forms of hCG. A strong positive correlation was seen between serum βhCG and urinary cßhCG in cancer patients (r = 0.703, P < 0.001). Similar correlations were observed between urinary βhCG and cßhCG (r = 0.665, P < 0.001) and between serum and urinary βhCG (r = 0.617, P = 0.001). The correlation between serum hCG and urinary cßhCG was not significant (r = 0.101, P = 0.654). In benign disease the levels of urinary cßhCG correlated with the serum (r = 0.674, P = 0.002) and urinary levels of hCG (r = 0.575 P = 0.025) and βhCG (r = 0.482, P = 0.006) but not with those of serum βhCG (r = 0.231, P = 0.190).

Table 3  Frequency of elevated values of hCG, βhCG, and cßhCG in urine of patients with malignant and benign pancreatic and hepatobiliary diseases
The cutoff levels are based on the 97.5 percentile of a reference group matched for age and sex.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>hCG (%)</th>
<th>βhCG (%)</th>
<th>cßhCG (%)</th>
<th>hCG + βhCG (%)</th>
<th>cßhCG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic cancer</td>
<td>29</td>
<td>1(3%)</td>
<td>8(28%)</td>
<td>16(55%)</td>
<td>11(38%)</td>
<td></td>
</tr>
<tr>
<td>Biliary cancer</td>
<td>7</td>
<td>0(0%)</td>
<td>4(57%)</td>
<td>5(71%)</td>
<td>3(43%)</td>
<td></td>
</tr>
<tr>
<td>Benign diseases</td>
<td>45</td>
<td>1(2%)</td>
<td>6(13%)</td>
<td>5(11%)</td>
<td>2(4%)</td>
<td></td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>14</td>
<td>0(0%)</td>
<td>2(14%)</td>
<td>0(0%)</td>
<td>1(7%)</td>
<td></td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>11</td>
<td>1(9%)</td>
<td>1(9%)</td>
<td>1(9%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Benign biliary obstruction</td>
<td>20</td>
<td>0(0%)</td>
<td>3(15%)</td>
<td>4(20%)</td>
<td>1(5%)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Concentrations of hCG, βhCG, and cßhCG in urine of patients with malignant and benign diseases. For details see Fig. 1.

Fig. 3. Follow-up of a patient with pancreatic cancer. The levels decreased after surgical removal of the tumor and started to increase 2 months before a recurrence was observed clinically.

Gel Chromatography of Serum and Urine. βhCG and cßhCG in two serum and four urine samples were characterized by gel chromatography. In serum both βhCG and cßhCG eluted at the same position as the corresponding purified substances. Mean recovery after gel chromatography was 138% (range, 99–169%). In two of four urine samples part of the cßhCG immunoreactivity (18–27%) eluted between βhCG and cßhCG, indicating an intermediate molecular size (Fig. 5). This component reacted weakly in the βhCG assay. The cßhCG immunoreactivity in serum eluted at the expected position (Fig. 6). The concentrations of cßhCG in these samples were 10, 27 and 38 pmol/liter, respectively, corresponding to 15%, 37%, and 17% of the βhCG level. In these patients the concentration of cßhCG in urine was 88–632 pmol/liter, and cßhCG accounted for 82–96% of total hCG immunoreactivity.

DISCUSSION

This study shows that patients with pancreatic cancer often have elevated concentrations of βhCG in serum and cßhCG in urine, and their concentrations are strongly correlated. In contrast, the concentrations of hCG in serum and urine are normal or only marginally elevated. cßhCG immunoreactivity also occurs in the serum of many cancer patients, but the levels are lower than those of βhCG. It has been shown that cßhCG is the main form of hCG found in urine after i.v. injection of purified βhCG, and cßhCG is thought to be formed by the proteolytic breakdown of βhCG in the kidneys (10, 36). Furthermore,
Fig. 4. ROC curve analysis of different forms of hCG in serum and urine as markers for pancreatic cancer. The reference group consisted of patients with pancreatitis (n = 25) and biliary obstruction (n = 20). A, curves for hCG, βhCG, and cβhCG in serum; B, curves for urine.

Fig. 5. Gel chromatography on Sephadex G-75 of urine from a patient with biliary cancer. βhCG and cβhCG in the fractions were quantitated by IFMA.

Fig. 6. Gel chromatography on Sephadex G-75 of serum from the patient in Fig. 5. βhCG (○) and cβhCG (●) in the fractions were quantitated by IFMA.

βhCG has been shown to be the major form of hCG immunoreactivity in tumor extracts and to be secreted by various tumor cells in vitro (37). These and the present results suggest that cβhCG in the urine of cancer patients is mainly derived from βhCG secreted by the tumor and that part of it appears to be formed before excretion into urine.

Characterization of cβhCG immunoreactivity in serum by gel chromatography indicated that it behaved as cβhCG purified from urine. This is in agreement with our finding for cβhCG in pregnancy serum (7). The cβhCG in pregnancy and chorionic cancer serum has been claimed to occur in a complex, which has to be dissociated and separated by gel chromatography before it can be detected (38). Our attempts to reproduce these findings have not been successful.5

In two of four urine samples analyzed by gel chromatography, part of the cβhCG immunoreactivity eluted with a molecular size between those of cβhCG and βhCG. However, the levels were low, which prevented further characterization. This component could be an intermediate degradation product of βhCG. Partially degraded forms of hCG have been demonstrated in the serum and urine of patients with chorionic cancer (39).

The median ratio of cβhCG to hCG in serum was 0.3. Because of this and the 37% cross-reaction of βhCG in the cβhCG assay the results for βhCG in serum are less reliable than those in urine. However, the gel chromatographic experiments confirmed the results of the direct assays. In urine the cβhCG concentrations were about 3-fold those of βhCG. Therefore, the cross-reaction had only a minor effect (5%) on the urine values. Cross-reaction of hCG in the βhCG assay (0.15%) was insignificant for interpretation of the results because the hCG concentrations were relatively low (<45 pmol/liter). The hCG assay probably does not detect βhCG at all, since the tracer antibody in this assay is directed toward the α subunit of hCG. The apparent cross-reaction of 0.6% is likely to be due to contamination of the βhCG preparation with hCG.

Because elevated levels of βhCG and cβhCG may occur in benign disease, we also compared the clinical performance of the various assays by ROC curve analysis using patients with benign diseases as a reference group. At cutoff levels giving 90% specificity the sensitivity of serum βhCG was 72% and that of urinary cβhCG was 59%. When the cutoff was lowered to give 75% specificity the sensitivity of both markers was 79%. This suggests that the sensitivity and specificity of serum βhCG and urinary cβhCG are comparable with those of CA 19-9 and carcinoembryonic antigen, which are established markers for pancreatic and biliary cancer. In a similar study, these markers were shown to have sensitivities of 54% and 78% at cutoff levels giving specificities of 76% and 78%, respectively (40).

In earlier studies showing elevated levels of hCG immunoreactivity in the serum of cancer patients, radioimmunoassays measuring both hCG and βhCG were used. On the basis of the shape of the dilution curves Braunstein et al. (18) drew the

conclusion that the immunoreactivity consisted of hCG. In our study, only four serum samples (14%) and one urine sample (3%) had elevated hCG levels, and the levels were only 2–3-fold the cutoff level and only 50% higher than the highest levels (9 IU/liter) occasionally observed in postmenopausal women (11). This finding is in agreement with the results of Özturk et al. (4). For comparison, the highest concentrations of βhCG were 120-fold and that of εβhCG 86-fold the cutoff level. Therefore, βhCG appears to be the major form of hCG causing elevated hCG immunoreactivity in the serum of patients with pancreatic and biliary adenocarcinoma, but intact hCG may be expressed by some tumors. Alternatively, βhCG produced by tumors could recombine with pituitary α subunit in serum. This possibility seems unlikely because recombination of α and β subunits usually requires much higher concentrations than observed in the present study (41).

In patients with endocrine pancreatic cancer, βhCG has been found to be the main form of hCG immunoreactivity in serum, but α subunit may also be produced (21, 22). Expression of βhCG is common in various tumors and cell lines (37), but βhCG in serum has not been found thus far to be a useful tumor marker (4). This has apparently been due to lack of sufficiently sensitive and specific assays. A high sensitivity is essential because of the low levels of βhCG in normal serum. The method used in the present study is 8–70-fold more sensitive than earlier ones (4, 15, 42). βhCG in serum was characterized by gel chromatography, which confirmed that the molecular size of immunoreactive material corresponded to that of purified βhCG. The mean recovery of βhCG after chromatography was 138%, suggesting that nonspecific interference by serum and urine components (43) caused a slight underestimation in the IFMA of βhCG.

We have also analyzed whether total hCG-like immunoreactivity, i.e., the sum of hCG, βhCG, and εβhCG in serum, could be used, rather than βhCG in serum or εβhCG in urine. This roughly corresponds to what is measured by competitive immunoassays, e.g., radioimmunoassay. The sensitivity of this assay method was 41% as compared with 72% for βhCG. The concentration of βhCG in postmenopausal serum is only 10–20% of the total hCG immunoreactivity, and in contrast to the levels of hCG those of βhCG increase only slightly with age. Because of the large range in hCG levels even a 3–5-fold increase in βhCG will not necessarily increase total hCG immunoreactivity above the cutoff level. Therefore, βhCG is superior to total hCG immunoreactivity as a serum marker for pancreatic cancer, whereas εβhCG is the best urine marker. In this study βhCG in serum was a better marker than εβhCG in urine, apparently because the latter can be derived not only from βhCG but also from hCG.

The elevation of βhCG in serum and εβhCG in urine in benign diseases is a new finding. It may indicate that expression of βhCG is not limited to neoplastic and trophoblastic cells but may also occur in nonmalignant cells in connection with inflammatory reactions or tissue regeneration. This would actually not be surprising, considering the fact that other tumor markers, e.g., CA 19-9, CA-50, and carcinoembryonic antigen, also show a similar elevation in benign diseases (40, 44). However, the elevation of βhCG in serum and εβhCG in urine is infrequent (9–11%) and moderate (2-fold) in comparison with 9–15-fold elevations and 16–35% elevated levels of CA 19-9, CA-50, and carcinoembryonic antigen in patients with benign pancreatic and biliary diseases (45, 46). Thus the elevation in benign disease does not invalidate the use of βhCG and εβhCG as tumor markers.

ACKNOWLEDGMENTS

Drs. Robert E. Canfield, John O’Connor, Steven Birken, and Alexander Krichevsky of the College of Physicians and Surgeons of Columbia University (New York, NY) are gratefully acknowledged for their kind gift of εβhCG and monoclonal antibodies. The expert technical assistance of Taina Grönholm, Maarit Leinimaa, and Anja Mäki is gratefully acknowledged.

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