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Combination Assay of Urinary β-Core Fragment of Human Chorionic Gonadotropin with Serum Tumor Markers in Gynecologic Cancers

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Ectopic production of the immunoreactive β-subunit of human chorionic gonadotropin (IR-hCGβ) by gynecologic malignancies has been well recognized, but IR-hCGβ has not yet been established as a clinically useful tumor marker, except for germ cell tumors. We measured the concentrations of IR-hCGβ-related molecules, intact hCG, free hCGβ, and β-CF, in the sera and urine of patients with various gynecologic cancers (cervical, endometrial, and ovarian cancers) to assess their clinical usefulness as a tumor marker in comparison with serum tumor markers such as CEA, SCC, CA125, and CA19-9. The highest incidence of IR-hCGβ was obtained in the assay for β-CF in the urine, with positive rates of 47.7% (94 of 197) for cervical, 37.8% (14 of 37) for endometrial, and 84.4% (38 of 45) for ovarian cancers with a cut-off value of 0.2 ng/mg of creatinine. In cervical cancer, there was no significant correlation between the concentrations of urinary β-CF and serum SCC, and 57.9% (114 of 197) of the patients were detected by the combination assay of these tumor markers. Serial determination in 22 cervical cancer patients with elevated urinary β-CF level prior to therapy showed that its level decreased after successful treatment, but 4 of 5 patients with persistent or recurrent disease had elevated levels of urinary β-CF. All of the ovarian cancer patients examined were detected by the combination assay of urinary β-CF and serum CA125. The levels of urinary β-CF showed little correlation with those of the serum tumor markers, indicating the usefulness of the combination assay of urinary β-CF with serum tumor markers for detecting cervical and ovarian cancers.

Key words: Tumor marker — Chorionic gonadotropin — Ectopic production — Gynecologic cancer — Urinary protein

Human chorionic gonadotropin (hCG), a glycoprotein composed of α- and β-subunits, is a highly sensitive marker of pregnancy and trophoblastic diseases.[1] Elevated levels of immunoreactivity of hCGβ-subunit (IR-hCGβ) have been found in the serum of some patients with a variety of nontrophoblastic tumors.[2,3] In particular, the high incidence of IR-hCGβ in patients with nontrophoblastic gynecologic malignancies is well recognized.[4,5] However, the relatively low incidence of elevated serum IR-hCGβ and the low titer have limited its value as a tumor marker, except for germ cell tumors. Recently, it has been observed that elevated levels of IR-hCGβ are found more frequently in the urine than in the serum of patients with nontrophoblastic tumors.[6–8] In addition, urinary IR-hCGβ mainly consists of hCGβ-related low-molecular-weight material, which is barely detected in the serum.[9] This material was termed hCGβ-core fragment (β-CF) because it retains the hCGβ-core conformational determinant recognized by an hCGβ-core antiserum such as SB6, but lacks the carboxy-terminal portion of hCGβ.[10] Previous studies have shown that elevated levels of β-CF are often found in the urine of patients with nontrophoblastic tumors, even when IR-hCGβ can not be detected in the serum. O'Connor *et al.*[11] and Cole *et al.*[12] developed immunoassays to measure urinary β-CF and indicated its potential value as a tumor marker for gynecologic cancers, but little work has been performed on its use in conjugation with other tumor markers. In this study, we measured the concentration of IR-hCGβ in the sera and urine of patients with gynecologic cancers and assessed its clinical value as a tumor marker in combination with serum tumor markers.

MATERIALS AND METHODS

Patients and samples This study included 197 patients with cervical, 37 with endometrial and 45 with ovarian cancers, and 207 with benign diseases (121 uterine myoma, 38 endometriosis, and 48 benign ovarian cyst). The urine and serum samples were collected on admission and then frozen at −20°C until assayed. To deter-

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mine the cut-off value, urine and serum samples were collected from 112 healthy nonpregnant women.

Enzyme immunoassay (EIA) for IR-hCGβ Three types of EIA to measure intact hCG (EIA-1), free hCGβ (EIA-2), and β-CF (EIA-3) were developed as previously described.[13] In all three EIAs, the sensitivity was 0.01 ng/ml and the cross-reactivities of pituitary glycoprotein hormones (hLH, hFSH and hTSH) were less than 0.5%. The urinary concentrations of intact hCG, free hCGβ, and β-CF were adjusted based on the concentration of creatinine in the same urine and expressed as nanograms per milligram of creatinine (ng/mgCr). EIA-1 and EIA-2 are essentially specific for intact hCG and free hCGβ, respectively, indicating that the assay values of EIA-1 and EIA-2 correspond to the actual concentrations of intact hCG and free hCGβ, respectively. The assay value of EIA-3, however, does not always indicate the concentration of β-CF, since EIA-3 recognizes β-CF as well as free hCGβ. Therefore, the actual amount of β-CF was calculated after subtracting the assay value in EIA-2 from that in EIA-3.

Gel chromatography on Sephadex G-100 The serum and urine samples containing large amounts of IR-hCGβ from 2 patients (F.T. and H.S.) with cervical squamous cell carcinoma, stage IIb, and a patient (T.I.) with ovarian serous adenocarcinoma, stage IIIc, were qualitatively analyzed by column chromatography on Sephadex G-100. Two ml of the serum and the urine were each applied to a column (1.6×90 cm) of Sephadex G-100 equilibrated with 0.05 *M* sodium phosphate buffer, pH 7.4, containing 0.15 *M* NaCl and 0.02% sodium azide. The flow rate of the column was adjusted to 10 ml/h and 2.7 ml fractions were collected at 4°C.

Serum tumor markers The positive rates of carcinoembryonic antigen (CEA), squamous cell carcinoma-related antigen (SCC), CA125, and CA19-9 were simultaneously investigated in the sera of the patients for comparison with that of urinary IR-hCGβ. The serum concentrations of CEA, SCC, CA125, and CA19-9, were measured by using an RIA-kit (Dynabott, Tokyo), an EIA-kit (Boehringer Mannheim, Mannheim), an IRMA-kit (Centocor, Malvern), and a Latex immunoassay (Toray, Tokyo), respectively. The cut-off values were 5.0 ng/ml for CEA, 1.5 ng/ml for SCC, 35 IU/ml for CA125, and 37 IU/ml for CA19-9.

RESULTS

Gel chromatography on Sephadex G-100 The elution profiles of the serum and urine samples from patients with cervical cancer and ovarian cancer are shown in Fig. 1. In all cases, there were remarkable quantitative and qualitative differences between serum and urinary IR-hCGβ. In the serum, a small but distinct peak of IR-hCGβ was detected at the elution position of free hCGβ by both EIA-2 and EIA-3. While EIA-2 is specific only for free hCGβ, EIA-3 recognizes β-CF as well as free hCGβ. Accordingly, the present results indicate that

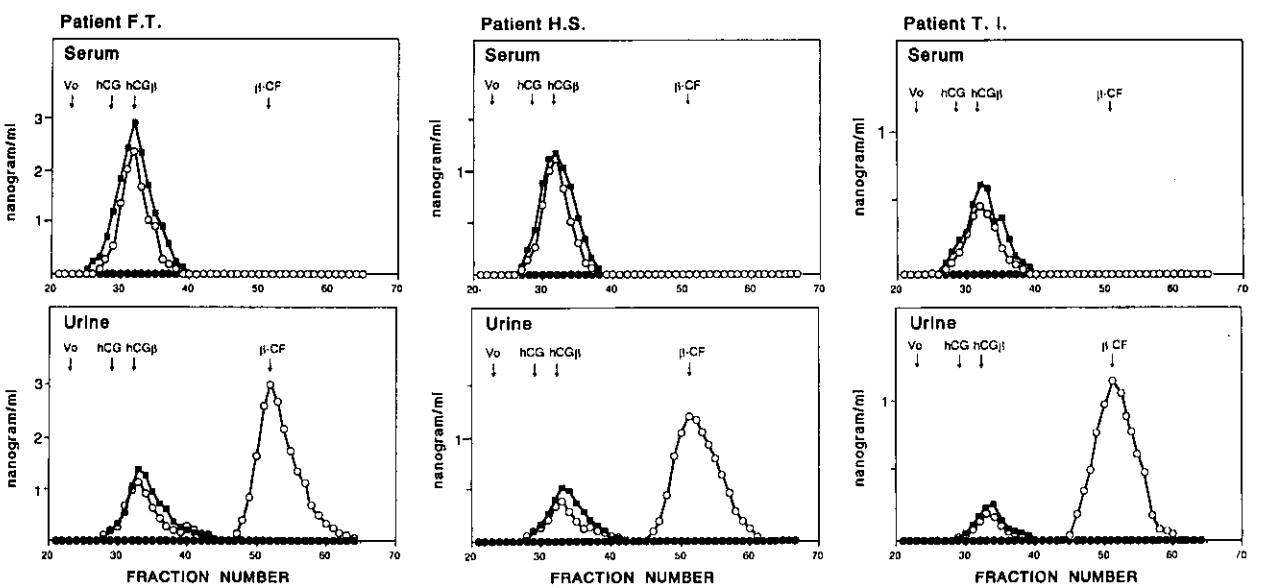

Fig. 1. Elution profiles of IR-hCGβ in the serum and urine of 2 patients (F.T. and H.S.) with cervical cancer and a patient (T.I.) with ovarian cancer by gel chromatography on Sephadex G-100. Eluted fractions were assayed by EIA-1 (●), EIA-2 (■), and EIA-3 (○) as described in "Materials and Methods." Arrows indicate void volume (Vo) and the elution positions of intact hCG, free hCGβ, and β-CF.

Table I. Sensitivity and Specificity of Measurements of Intact hCG, Free hCGβ, and β-CF in the Sera and Urine of Normal Women and Patients with Gynecologic Diseases

	Intact hCG		Free hCGβ		β-CF	
	Serum	Urine	Serum	Urine	Serum	Urine
Specificity (true negatives/all without disease, %)						
Normals (n=112)	96.4	96.4	98.2	97.3	95.5	94.6
Benign diseases						
Uterine myoma (n=121)	95.0	95.0	97.5	96.7	97.5	97.5
Endometriosis (n=38)	92.1	86.8	92.1	89.5	94.7	92.1
Ovarian cyst (n=48)	89.6	89.6	97.9	97.9	95.8	91.7
Sensitivity (true positives/all with diseases, %)						
Cancers						
Cervical (n=197)	14.2	15.2	18.3	15.2	1.0	47.7
Endometrial (n=37)	13.5	21.6	18.9	13.5	0	37.8
Ovarian (n=45)	26.7	31.1	55.6	37.8	2.2	84.4

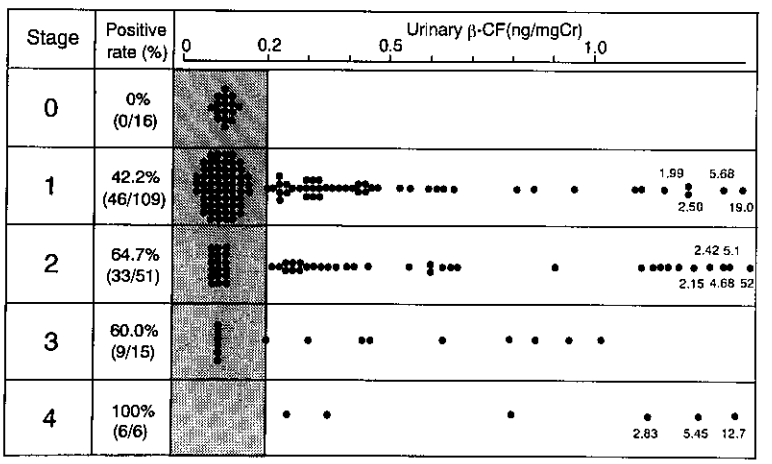

Fig. 2. Urinary β-CF levels according to stages of cervical cancer. The cut-off level of urinary β-CF was 0.2 ng/mgCr.

IR-hCGβ in the serum from these 3 patients is predominantly free hCGβ but not intact hCG or β-CF. On the other hand, in the urine, a large peak was detected at the elution position of β-CF only by EIA-3, in addition to a small peak of free hCGβ by EIA-2 and EIA-3. Intact hCG was not detectable by EIA-1 in any of the fractions. Furthermore, similar elution profiles were also observed in all the samples from 5 cases with cervical cancer and 3 cases with ovarian cancer (data not shown).

Cut-off values of IR-hCGβ in serum and urine The mean levels plus or minus standard deviation (M±SD) of intact hCG, free hCGβ, and β-CF in the sera of healthy women were 0.053±0.037, 0.025±0.013, and 0.028±0.017 ng/ml, respectively, and those in the urine were 0.050±0.036, 0.046±0.033, and 0.079±0.052 ng/mgCr, respectively. The cut-off values, set at M+2SD, of intact hCG, free hCGβ, and β-CF in the serum were 0.2, 0.1, and 0.1 ng/ml, respectively, and those in the urine were 0.2, 0.1, and 0.2 ng/mgCr, respectively. Based on these cut-off values, 3.6, 1.8, and 4.5% of the sera and 6.3, 2.7, and 5.4% of the urine samples from 112 healthy women were found to contain elevated levels of intact hCG, free hCGβ, and β-CF, respectively. Specificity of IR-hCGβ as a tumor marker was investigated by examining sera and urine of patients with gynecologic benign diseases as

Table II. Incidence of Elevated Levels of Serum Tumor Markers in Patients with Cervical, Endometrial, and Ovarian Cencers

	SCC	CEA	CA125	CA19-9
Cervical (n=197)	35.0%	25.4%	6.6%	9.1%
Endometrial (n=37)	5.4%	13.5%	18.9%	37.8%
Ovarian (n=45)	8.9%	15.6%	88.9%	40.0%

listed in Table I; the false-positive rates of IR-hCGβ were less than 10% in most of the benign diseases.

IR-hCGβ and serum tumor markers in patients In the sera of patients with cervical cancer, only 18.3% (36 of 197) were found to contain elevated levels of IR-hCGβ due to free hCGβ (Table I). By contrast, in the urine of these patients, elevated levels of IR-hCGβ, mainly accounted for by β-CF, were detected in 47.7% (94 of 197). The positive rates of serum SCC and serum CEA were 35.0% (69 of 197) and 25.4% (50 of 197), respectively, in the patients with cervical cancer (Table II). When the positive rate of urinary β-CF was compared with that of serum SCC, elevated levels of urinary β-CF were found in 42.2% and 64.7% of the patients at stage 1 and stage 2 (Fig. 2), respectively, whereas the positive rates of serum SCC were 22.9% and 51.0%, respectively. Though the incidence of serum SCC was apparently higher in squamous cell carcinoma than in adenocarcinoma, as previously reported,[14] the positive rates of urinary β-CF were 46.2% (80 of 173) in squamous cell carcinoma and 50.0% (12 of 24) in adenocarcinoma, indicating that the incidence of urinary β-CF may be

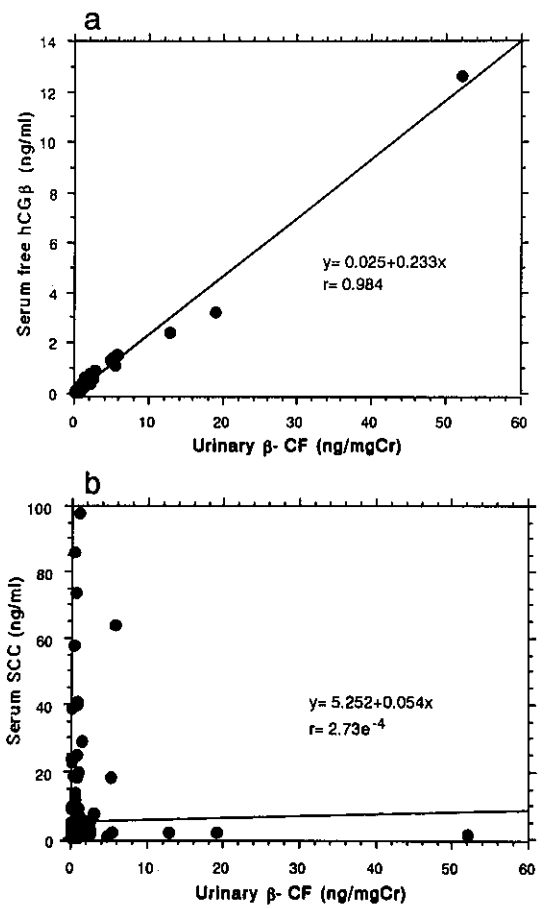

Fig. 3. Correlations of the levels of urinary β-CF and serum free hCGβ (a) and of urinary β-CF and serum SCC (b).

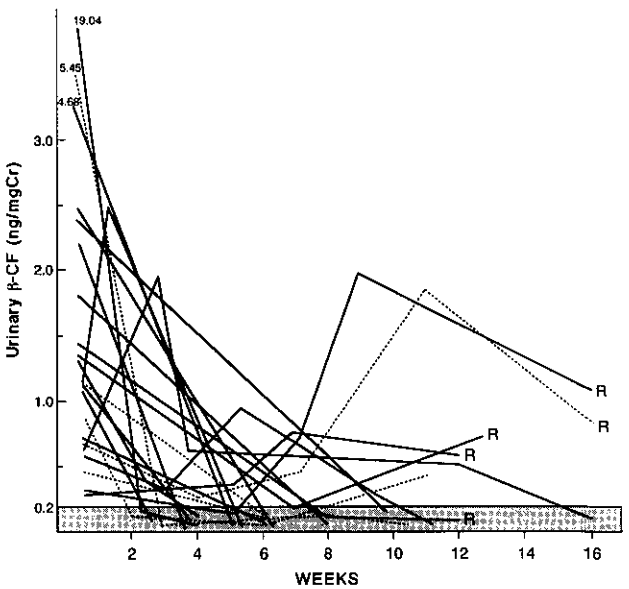

Fig. 4. Serial determination of urinary β-CF in 22 cervical cancer patients with elevated levels of urinary β-CF prior to therapy. Solid and dotted lines indicate the patients who received surgery and radiotherapy, respectively. R: recurrence.

Table III. Combination Assays of Urinary β-CF with Serum Tumor Markers

		SCC[a]			CA19-9[b]			CA125[c]		
		+	−	Total	+	−	Total	+	−	Total
β-CF[d]	+	47	45	92	5	9	14	33	5	38
	−	22	83	105	9	14	23	7	0	7
Total		69	128	197	14	23	37	40	5	45

a) SCC measured in the serum of 197 patients with cervical cancer.
b) CA19-9 measured in the serum of 37 patients with endometrial cancer.
c) CA125 measured in the serum of 45 patients with ovarian cancer.
d) β-CF measured in parallel in the urine of the above patients.

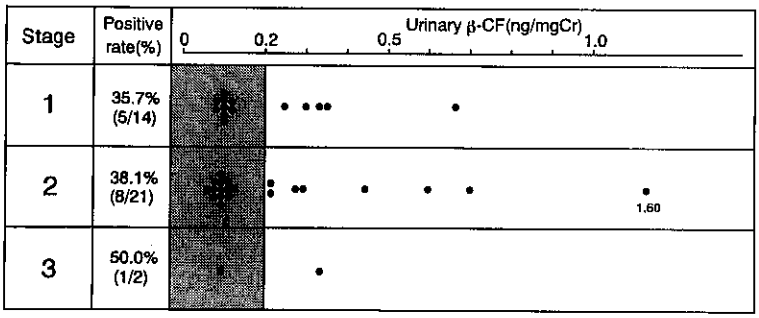

Fig. 5. Urinary β-CF levels according to stages of endometrial cancer. The cut-off level of urinary β-CF was 0.2 ng/mgCr.

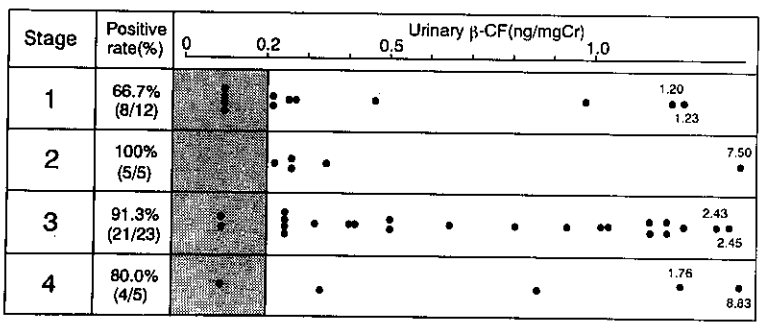

Fig. 6. Urinary β-CF levels according to stages of ovarian cancer. The cut-off level of urinary β-CF was 0.2 ng/mgCr.

independent of cell type. There was no significant correlation between the concentrations of urinary β-CF and serum SCC (Fig. 3), and 57.9% (114 of 197) of the patients were detected by the combination assay of these tumor markers (Table III). Serial determination of urinary β-CF was performed in 22 cervical cancer patients with elevated urinary β-CF level prior to therapy (Fig. 4). Between 3 and 8 weeks after successful treatment, the levels of urinary β-CF fell below the cut-off value (0.2 ng/mgCr). Four of 5 patients with persistent or recurrent disease were found to have elevated levels of urinary β-CF. In these patients, elevations of urinary β-CF were noted at least 1 month before the diagnosis based on clinical examinations.

The positive rate of urinary β-CF in endometrial cancer was 37.8% (14 of 37). However, the clinical usefulness of urinary β-CF as a tumor marker for endometrial cancer seemed to be poor because the elevated levels were still low (Fig. 5).

Of 45 patients with ovarian cancer, 38 (84.4%) patients had elevated levels of β-CF in their urine, and the incidence increased with disease progression (Fig. 6). In the sera of these patients, the positive rates of CA125, CA19-9, CEA, and SCC were 88.9, 40.0, 15.6, and 8.9%, respectively. It was striking that all of the patients with ovarian cancer so far examined were detected by the combination assay of urinary β-CF with serum CA125 (Table III). The positive rates of urinary β-CF classified by histologic types of ovarian cancer were 87.1% (27 of 31) for serous, 75.0% (6 of 8) for mucinous, and 83.3% (5 of 6) for others, while those of CA125 were 93.5% (29 of 31), 50.0% (4 of 8), and 83.3% (5 of 6), respectively. Elevated levels of free hCGβ were also found in the sera from 25 (55.6%) of 45 patients and there was an extremely high correlation between the levels of serum free hCGβ and urinary β-CF (Fig. 7).

DISCUSSION

Ectopic production of IR-hCGβ by gynecologic malignancies has been well recognized and previous studies have shown that 18–36% of patients with cervical cancer had elevated levels of IR-hCGβ in their sera.[1] The present study also showed that some patients with gynecologic cancers have elevated serum levels of IR-hCGβ, which could be predominantly attributed to the presence of free hCGβ. When the serum free hCGβ was evaluated as a tumor marker, the positive rates were 18.3% for cervical, 18.9% for endometrial, and 55.6% for ovarian cancers. However, the elevated levels of serum free hCGβ were still low, limiting its value as a tumor marker for these cancers.

Elevated levels of IR-hCGβ were detected more frequently in the urine than in the parallel sera of patients with gynecologic cancers. Gel chromatography indicated that urinary IR-hCGβ was almost exclusively β-CF, which was barely detected in the serum. The positive rates of urinary β-CF were 47.7% (94 of 197) for cervi-

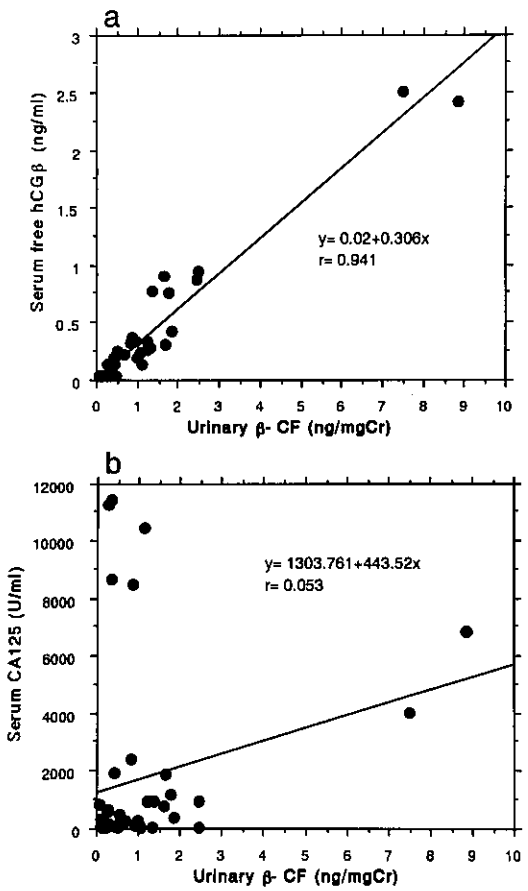

Fig. 7. Correlations of the levels of urinary β-CF and serum free hCGβ (a) and of urinary β-CF and serum CA125 (b).

cal, 37.8% (14 of 37) for endometrial, and 84.4% (38 of 45) for ovarian cancers; these rates are equal or superior to those of the tumor markers (SCC, CEA, CA125, and CA19-9) simultaneously measured in parallel sera.

SCC is a valuable tumor marker specific for squamous cell carcinoma of the cervix.[14] In the present study, the overall positive rate of serum SCC for cervical cancer was 35% (69 of 197), which was lower than that of urinary β-CF. In particular, the positive rates of urinary β-CF (42.4% in stage 1 and 64.7% in stage 2) were higher than those of serum SCC (22.9% and 51.0%, respectively), suggesting that urinary β-CF may be a useful marker for cervical cancer at the early stage. However, it is unfavorable as a tumor marker in that the degree of increase of urinary β-CF was considerably lower than that of serum SCC. There was no significant correlation between the concentrations of β-CF and serum SCC (Fig. 3), indicating the clinical usefulness of the combination assay of these markers.

For epithelial ovarian cancer, CA125 is a useful tumor marker.[15] In this study, 88.9% (40 of 45) of the patients with ovarian cancer were found to have elevated levels of serum CA125. In the urine of these patients, elevated levels of β-CF were detected in 84.4%. Histologically, it was noteworthy that elevated levels of urinary β-CF were detected in 75% (6 of 8) of the patients with mucinous adenocarcinoma, which is the only histologic subtype with a low incidence of abnormal levels of serum CA125. In addition, the positive rate of urinary β-CF was only 7.9% (3 of 38) for endometriosis, which elevates serum CA125 levels.

For endometrial cancer, there is no established tumor marker such as SCC for cervical cancer or CA125 for ovarian cancer. Cole *et al.*[12] reported that 76.9% (10 of 13) of the patients with endometrial cancer had elevated levels of IR-hCGβ in the urine. In the present study, however, 37.8% (14 of 37) were found to have only weakly elevated levels of urinary β-CF, indicating that it is of little value as a tumor marker.

The mechanism of excretion of β-CF into the urine is unknown. The previous studies on the structure of β-CF strongly suggested that it is a proteolytic product of the β-subunit of hCG.[16,17] We found an extremely high correlation between the concentrations of urinary β-CF and serum free hCGβ in the patients, indicating that β-CF may be a degradative product of free hCGβ. In addition, we[18] analyzed IR-hCGβ in the sera and urine of rats and mice transplanted with human bladder cancer producing free hCGβ and deduced that the free hCGβ may be a precursor of urinary β-CF. Whatever the origin of the elevated levels of β-CF in the urine, it might be a potential tumor marker for a variety of cancers, including cervical, ovarian, lung, and bladder cancers.

In summary, we have shown here that elevated levels of IR-hCGβ were detected in the urine much more frequently than in the parallel sera of patients with gynecologic cancers. The urinary IR-hCGβ was mainly β-CF, which was barely detected in the serum. The levels of urinary β-CF showed little correlation with those of the serum tumor markers such as SCC, CEA, CA125, and CA19-9. Thus, we conclude that combination assay of urinary β-CF with serum tumor markers would be useful for identifying patients with cervical and ovarian cancers and for monitoring their response to treatment.

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