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Epidermal growth factor in urine from patients with bladder cancer

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Abstract Epidermal growth factor (EGF), a mitogenic polypeptide with a molecular weight of 6000, is excreted in human urine in nanomolar quantities. Recently, some reports showed that urothelial neoplasm was related to the concentration of EGF in urine. In this study, EGF concentration in urine was measured by enzyme-linked immunosorbent assay (ELISA) in 207 samples from 112 male patients (30–90 years old, median 66.2) who had previously been treated for bladder cancer. Then, we tried to clarify the significance of urinary EGF as a marker for recurrence of bladder cancer in comparison with urine cytology. The samples were collected on occasional follow up cystoscopy. Urine from nine age-adjusted males without urological disease was also measured to obtain normal control values. In 123 samples from patients without tumors, EGF concentrations in urine decreased with age. In 84 samples obtained from patients with recurrent tumor, EGF concentrations were significantly lower than those in 123 samples from patients without tumors ($P < 0.001$) Furthermore, EGF concentrations in longitudinal samples collected the same patients during tumor recurrence and at the times when no tumor was detected were measured in 56 patients. EGF concentrations in the samples collected during tumor recurrence was significantly lower than that in specimens collected when there was no tumor ($P < 0.01$). There were no significant differences between the same samples collected during tumor recurrence with regard to tumor grade, stage shape and number of tumors. However, EGF concentration in urine from patients with carcinoma in situ (CIS) was lower than that in specimens from patients without CIS. These results indicate the usefulness of determining the EGF concentration as a marker for detecting bladder cancer recurrence. Urine cytology was also examined in the same series and findings were compared with those of urinary EGF. On cytology, class IV and V were considered positive, and on urinary EGF, less than l0 ng/mgCr were considered positive. Sensitivity was 25% for cytology and 57% for urinary EGF, while specificity was 98% and 66%, respectively. The predictive positive value was 0.88 and 0.53, respectively. With the combined use of urinary EGF and cytology, the sensitivity, specificity and predictive positive value were 68%, 64% and 0.92, respectively. In conclusion, urinary EGF seems to be a useful marker for detecting bladder cancer recurrence if performed in addition to cytology.

Key words Bladder cancer · Epidermal growth factor · Tumor marker

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Introduction

Epidermal growth factor (EGF), a mitogenic polypeptide with a molecular weight of 6000, is excreted in human urine in nanomolar quantities. EGF has been found to have co-carcinogenic properties, and to be required for the promotion of chemically initiated tumors in some systems [6, 13]. The action of EGF is mediated by binding to a specific membrane-bound receptor (EGFr) which has a close structural relationship with the oncogene product of *erb*-B of the avian erythroblastosis virus [2]. Recently, increased levels of EGFr protein have been identified in urothelial cancer [8, 10, 11, 14], and amplification of the EGFr gene has occasionally been identified in transitional cell carcinoma (TCC) [3, 7]. Consequently, EGF in the urine of patients of urothelial cancer may bind to the overly expressed EGFr, and thus may be excreted in low concentrations. Some reports [4, 5, 9] showed that urothelial neoplasm may be related to a low concentration of EGF in urine. However, those studies involved a relatively small number of patients and details were unclear in some reports. In this

study, EGF concentration in urine from bladder cancer patients was measured and data were compared with urine cytology findings to clarify the significance of urinary EGF as a marker for bladder cancer recurrence.

Materials and methods

Urinary EGF concentration was measured in 207 samples from 112 male patients (30–90 years old, median 66.2) who had previously been treated for bladder cancer (background of the patients is shown in Table 1). The samples were collected on the occasional follow up cystoscopy at our outpatient clinic. Eighty-three of the samples were collected at the time of tumor recurrence and 123 of these samples were collected when no recurrent tumors could be detected. Urine samples from age-adjusted males without urological disease were also measured to obtain normal control values.

A 10 ml sample from each patient was frozen at −80 °C for subsequent analysis. Urinary EGF was measured by enzyme-linked immunosorbent assay (ELISA), essentially described previously by Nitta et al. [12]. Briefly, 96-well plates with anti-human EGF antibody were used. The wells were washed and standards or samples were diluted 50 times and added to the wells. These were incubated overnight at room temperature and then the wells washed. Rabbit-anti-hEGF-serum was added to the wells that were then incubated for 2 h at room temperature. These were washed again and POD-labeled goat- anti-rabbit-IgG was added to the wells. After a further incubation for 2 h at room temperature, OPD solution was added. Then, the level of hEGF was counted at 492 nm; the range of measurable hEGF by this assay was 1–100 pg/well. Recoveries of hEGF added to serum and urine ranged between 80 and 90%. The intra- and inter-assay coefficients of variation were less than 7 and 9%, respectively.Then urinary creatinine was also measured in each sample, and the urinary EGF/creatinine ratio was used for this study.

Urine cytology was investigated in the same series, and findings were compared with the data on urinary EGF. On cytology, class IV and V were considered positive, and on urinary EGF, less than l0 ng/mgCr was considered positive based on the findings of this study and prior studies. In a combination analysis, when urine cytology and/or urinary EGF were positive, the result was considered positive. Statistical analysis was performed using the Wilcoxon test.

Table 1 Patients' background

Data	Number
Patient	
Male	112
Age	34–90 (Ave. 66.2)
Samples	
Rec. (−)	123
Roe. (+)	84
Grade (onset)	
G1	24
G2	42
G3	37
Gx	9
Stage (onset)	
Ta	40
T1	45
T2	7
Tis	4
Tx	16
No. of tumors	
Solitary	48
Multiple	48
Unknown	15

Results

In 123 samples from patients without tumors, urinary EGF concentration showed a tendency to decrease with increasing age, as reported by Uchihashi et al. [15].

In 84 samples from patients with recurrent tumor, EGF concentrations (mean 10.07 ± 7.30 ng/mgCr) were significantly lower ($P = 0.0002$) than those in the samples from patients without tumors (mean 14.10 ± 7.64 ng/mgCr) (Fig. 1) and from normal control (mean 14.50 ± 8.60 ng/mgCr). There was no significant difference in age distribution between these two groups.

Furthermore, in 56 patients, urinary EGF concentrations in the samples collected during tumor recurrence (mean 9.70 ± 6.92 ng/mgCr) were significantly lower ($P = 0.004$) than those of specimens obtained at times without tumors (mean 14.07 ± 7.72 ng/mgCr). For every case except 6 (89.3%) of tumor occurrence as compared with cases that did not exhibit any tumor occurrence, there was a decreasing or stability of urinary EGF level (Fig. 2).

Urinary EGF concentrations showed a tendency to increase with tumor differentiation; for all other situa-

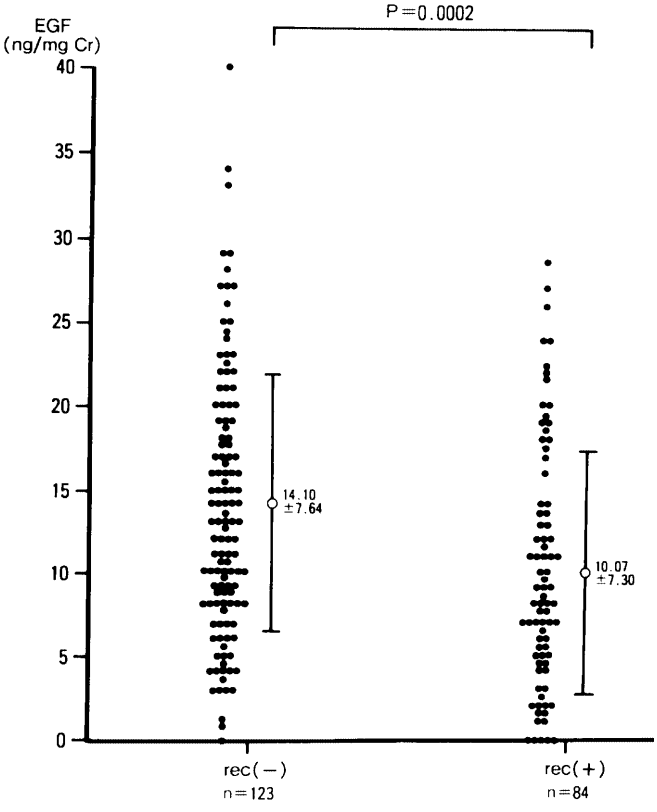

Fig. 1 Urinary concentration of EGF between recurrent samples and non-recurrent samples

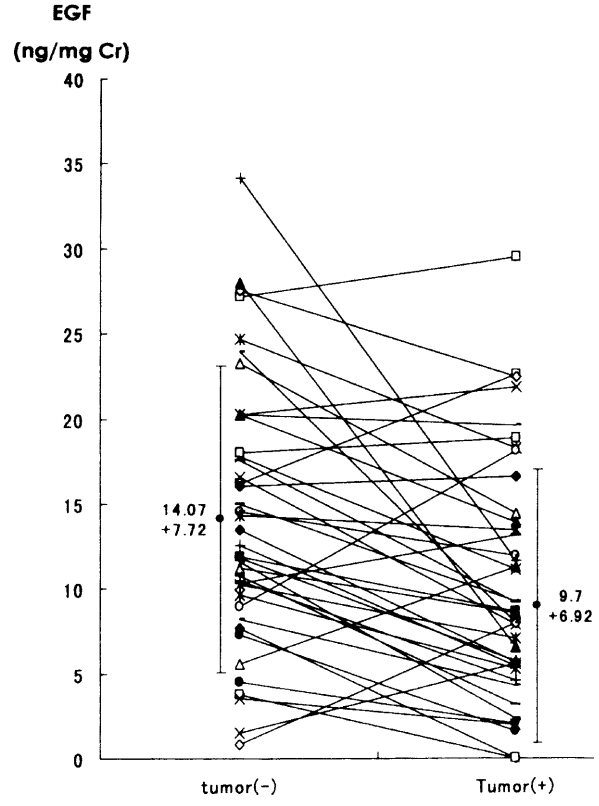

Fig. 2 Urinary concentration of EGF between recurrent and non recurrent samples in same cases ($n = 56$)

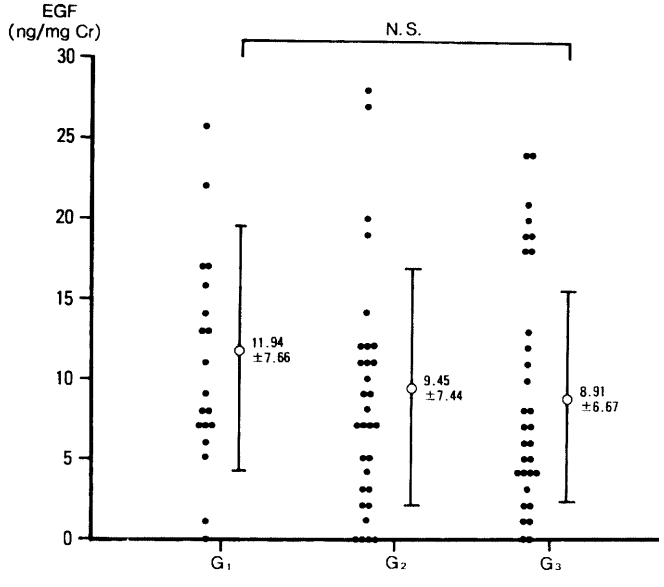

Fig. 3 Urinary concentration of EGF with tumor grade

tions any changes detected were not found to be statistically significant (Fig. 3). Urinary EGF concentrations showed a tendency to decrease with tumor infiltration (Fig. 4). Tumor shape and number of tumors did not affect the EGF concentration (data not shown). However, in patients with carcinoma in situ (CIS), urinary

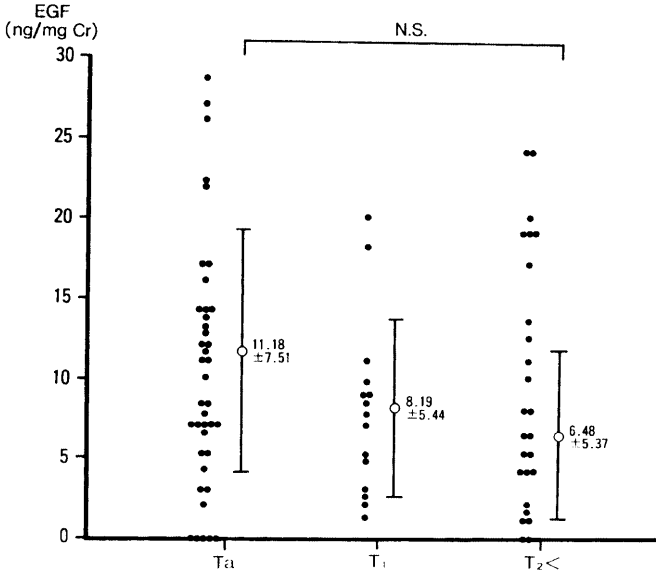

Fig. 4 Urinary concentration of ECF with tumor stage

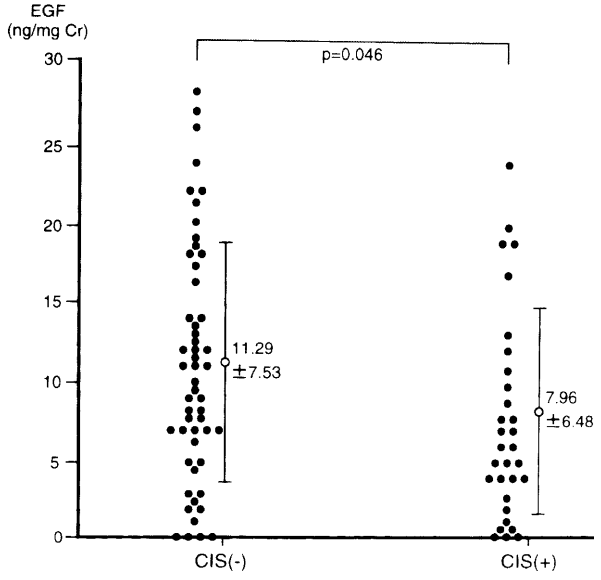

Fig. 5 Urinary concentration of EGF between tumor with and without CIS

EGF concentrations (mean 7.96 ± 6.48 ng/mgCr) were significantly lower ($P = 0.046$) in comparison to those of specimens from patients without CIS (mean; 11.29 ± 7.53 ng/mgCr) (Fig. 5).

The results of urine cytology and data on urinary EGF are shown in Tables 2 and 3, respectively. The results of combining cytology and urinary EGF findings are shown in Table 4.

Findings of urine cytology were compared with those of urinary EGF. Sensitivity was 25% for cytology and 57% for urinary EGF, while specificity was 98% and 66%, respectively. The positive predictive values were 0.88 and 0.53, respectively. With the combined use of urinary EGF and cytology, the sensitivity, specificity

Table 2 Urine cytology

	Class IV, V	Class III≥	Total
Tumor (+)	21td	63	84
Tumor (−)	3	120	123
Total	24	183	207

Table 3 Urine EGF

	< 10.0 ng/Cr	≥10.0 ng/Cr	Total
Tumor (+)	48	36	84
Tumor (−)	42	81	123
Total	90	117	207

Table 4 Combination of urine cytology and EGF

	Positive	Negative	Total
Tumor (+)	57	27	84
Tumor (−)	44	79	123
Total	101	106	207

and positive predictive values were 68%, 64% and 0.92, respectively (Table 5).

Table 5 Combined use of urinary EGF and cytology

	Cytology	EGF	Combination
Sensitivity	25.0	57.1	68.0
Specificity	97.6	65.9	64.0
Validity Score	22.6	23.0	42.0
Positive Predictive value	0.88	0.53	0.92

Discussion

One of the major problems of superficial bladder cancer is frequent tumor recurrence. Generally, cystoscopic examination is performed every 3–4 months to detect tumor recurrence. Cystoscopic examination is a reliable method of detecting intravesical recurrence, but it is too painful to perform frequently. Transabdominal ultrasonography is a noninvasive method but is not very reliable especially for carcinoma in situ (CIS); urine cytology does not have a very high sensitivity. Therefore, a new marker for detecting tumor recurrence has been sought. In this study we have tried to determine the significance of urinary EGF as a marker for bladder cancer recurrence. EGF concentration in urine from bladder cancer patients was measured and data were compared with urine cytology.

High concentrations of EGF are excreted in urine and are known to play an important role in tumor promotion and growth in certain natural and experimental tumor systems [6, 13]. Consequently, EGF may well be a major stimulator of uroepithelial tumorgenesis. Our results and those from other reports [1, 45, 9] showed a lower concentration of EGF in urine from a patient with transitional cell carcinoma (TCC) when compared with patients without TCC. They interpreted these results to mean that the low concentration of urinary EGF could be related to binding to EGF recapture as demonstrated in neoplastic urothelial epithelium. Neal et al. [10] showed a higher positive level for EGF receptors in poorly differentiated tumors as compared with moderately differentiated tumors and a higher positive level for EGF receptors in invasive tumors than in superficial tumors. Our results may support this speculation regarding the low concentration and the urine EGF/urothelial EGF interaction in the biological behavior of bladder cancer patients, because urinary EGF showed a tendency to increase with tumor differentiation and decrease in patients with invasive tumors; nevertheless no significant difference could be attached to these data. Urine EGF may not be an independent prognostic factor for bladder cancer as Chow et al. mentioned [1]. On the other hand, as a marker for tumor recurrence, urine EGF examination is a cheap, easy and harmless method ELISA of EGF is stable and enables many samples to be examined collectively. Furthermore, in our results, urinary EGF in samples from patients with recurrent tumors was significantly lower than in samples from patients without tumors, although there was no significant difference in age distribution between these two groups. EGF concentrations in longitudinal samples collected from the same patient during tumor recurrence and at time when no tumor was detected were measured in 56 patients. EGF concentrations in the samples collected during tumor recurrence was significantly lower than that in specimens collected when there was no tumor. Moreover, 90% of the cases showed decreasing or stability of urinary EGF when they had tumor recurrence in comparison with when they had no tumor recurrence. These results suggest the potential usefulness of determining the urinary EGF concentration as a marker for detecting bladder cancer recurrence.

Our findings on the EGF concentration in urine as a marker for bladder cancer recurrence have shown that the method is valid, when combined with urine cytology. However, there are still a few problems with this method. Firstly, since specificity and positive predictive value of urinary EGF is relatively low if used independently, it should be combined with cytology. Secondly, urinary EGF concentration showed an age-related decrease and the normal range might vary in relation to age. One possibility for overcoming this clinically is to closely monitor the value of urinary EGF on serial examinations and if the value decreases significantly, the change could suggest tumor recurrence. Thirdly, our study was performed in male patients only because the urinary EGF concentration in women is higher compared with that in men. In the future we need to determine the normal range for females.

These problems will be resolved with further investigation; moreover determining the urinary EGF

concentration is an easy, non-invasive and efficient method for monitoring tumor recurrence. Therefore, determining the urinary EGF concentration is an easier method of following bladder cancer patients in an outpatient clinic. Since the test is so easy, it can be performed frequently just like urine cytology.

In our results, a high predictive positive value was shown for the combined use of urinary EGF and the cytology. This may encourage the use of this method as the main technique for monitoring bladder preserved patients. For example, if results are positive for EGF and/or urine cytology, then cystoscopic examination should be performed to confirm tumor recurrence.

In conclusion, urinary EGF seems to be a useful marker for detecting bladder cancer recurrence if performed in tandem with cytology.

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