1110-5704
African Journal of Urology

Vol. 7, No. 1, 2001
20-26

EVALUATION OF THE PROGNOSTIC VALUE OF THE EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTORS IN BLADDER CANCER

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KEY WORDS: bladder cancer, epidermal growth factor receptors, tumors markers

ABSTRACT

The present study was carried out to evaluate the role and prognostic value of the expression of epidermal growth factor receptors (EGFR) in serum and urine for the detection of human bladder cancer. The study comprised 30 patients with newly diagnosed transitional cell carcinoma of the bladder and 10 normal volunteers. The patients' age ranged from 42 to 76 years. The serum and urinary EGFR levels were evaluated using the ELISA technique. An indirect haemoagglutination (IHA) test was used for the detection of bilharzial antibodies. Cystoscopy, tumor resection and bimanual examination under anaesthesia were carried out for every patient. The patients were divided into 4 groups: Group A: 10 healthy individuals serving as a control group, Group B: 7 patients with grade I bladder cancer, Group C: 10 patients with grade II bladder cancer and Group D: 13 patients with grade III bladder cancer. Bilharziasis was recorded in 33 patients, 6 of them were in the control group. A comparison between the bilharzial and non-bilharzial patients did not reveal any significant difference in the serum EGFR expression (46.47±32.23 and 36±38.22 fmol/ml, respectively) nor in the urinary EGFR expression (45.42±29.78 and 41±31.16 fmol/ml, respectively). None of the patients with grade I bladder cancer was found to have stage T3 and T4 cancer, while all the patients with grade II and grade III cancer had invasive cancer (>Ta). The mean values of urinary and serum levels of EGFR in the control group were 12.37±7.21 and 11.9±8.17 fmol/ml, respectively, while the mean values of urinary and serum levels of EGFR in the cancer patients were 46.47±32.23 fmol/ml and 45.4±29.78 fmol/ml, respectively, which represents a significant increase in the serum and urinary EGFR in the cancer patients as compared to the control group. In addition, a stepwise increase in the serum and urinary EGFR was noted with the cancer grade increasing from grade I to III. The best cut-off values for serum and urinary EGFR were 22 fmol/ml and 25 fmol/ml, respectively. The best cut-off point for the serum EGFR level had a sensitivity of 83.3%, a specificity of 90% and an accuracy of 85%, while the best cut-off point for the urinary EGFR level had a sensitivity of 80%, a specificity of 90% and an accuracy of 82.5%. The results of the present study suggest that high levels of EGFR in the serum and urine of patients with bladder cancer are related to various malignant histological features (tumor grade) and invasiveness of bladder cancer. In the future, the evaluation of urinary and serum EGFR might help in the prediction of recurrence rate, response to systemic treatment and in the screening of bladder tumors.

Table 1: *Different Stages of Bladder Cancer in Relation to the Tumor Grades*

	Ta	T1	T2	T3	T4
Grade I	2	3	2	0	0
Grade II	0	2	6	2	0
Grade III	0	2	4	4	3

INTRODUCTION

Human bladder tumors demonstrate a heterogeneous biological behaviour. Epidemiological studies of bladder cancers have suggested that they have different growth potentials[1]. Depite the emergence of various prognostic markers, it is difficult to predict tumour progression precisely. The classical clinicopathological staging has not proved to be effective in the prediction of which superficial tumors will lead to infiltration and which infiltrating tumors will metastasize. The ability to define high-risk patients would enable us to choose between conservative and radical treatment at an early stage and would help in developing a multimodal therapy in advanced disease[2].

Recently, some attention has been drawn to the growth factors. Due to its binding to the membrane receptor, the epidermal growth factor (EGF) stimulates the growth in a wide variety of cell types[3]. The expression of epidermal growth factor receptors (EGFR) has a significant homology with the C-erb B oncogene product[2]. Osborne et al.[4] emphasized that the amount of EGFR expressed by the tumor could reflect its growth potential and clinical aggressiveness. The gene for EGFR is located on chromosome 7; triosomy 7 is also associated with bladder cancer, particularly with more aggressive tumors[5].

EGFR is a transmembrane glycoprotein which has tyrosine kinase activity and augments cell proliferation on interaction with its ligant EGF[6]. Under normal circumstances, the EGFR is confined to the basal layer of the epithelial cells. In both transitional and squamous cancer, however, the EGFR becomes expressed on cells of all layers including those of luminal surfaces[7]. EGFR is expressed at an increased level in certain carcinomas. High levels of EGFR have been detected in squamous cell carcinoma of the lung, head and neck[8]. The presence of an increased EGFR level has been useful in distinguishing epidermoid cancer from small cell and adenocarcinomas. In addition, EGFR expression has been found to have a good prognostic and diagnostic role in brain tumors, esophageal cancer, cervical and ovarian tumors[9-11].

The expression of EGFR has recently been suggested to also play an important role in the progression of bladder cancer through paracrine stimulation of EGF[12]. Neal et al.[13] demonstrated that an abnormal expression of EGFR in bladder cancers is an independent predictor of poor survival. The abnormal distribution of EGFR is also seen in the dysplastic and normal appearing urothelium, both near-by and remote from transitional cell cancer[7].

In the present study we compared the degree of EGFR expression with tumor grades and stages of bladder cancer and evaluated the possibility of using it as a prognostic indicator and a marker for malignant disease.

PATIENTS AND METHODS

This study comprised 30 patients with newly diagnosed transitional cell carcinoma of the bladder admitted to the Urology Department of Tanta University Hospital for transurethral resection or biopsy. In addition, 10 normal volunteers were included in the study serving as a control group. All patients were subjected to thorough clinical examination and laboratory investigations including urine analysis, kidney function tests, indirect haemoagglutination (IHA) for the detection of bilharzial antibodies, total urinary and serum protein and evaluation of the EGFR level in serum and urine.

All patients underwent cystoscopy, bimanual examination under anaesthesia, tumor resection and, when indicated, computer tomography scans. The tumors were staged by the TNM system and graded histologically according to the system described by Hemanek and Sobin in 1987[4]. The patients were categorized into the following four groups: Group A: 10 healthy volunteers (control group), Group B: 7 patients with grade I bladder cancer, Group C: 10 patients with grade II bladder cancer and Group D: 13 patients with grade III bladder cancer.

Table 2: Level of EGFR Expression of the Different Groups in Serum and Urine

Group	SerumEGFR	Urinary EGFR	P*
Group A	12.37±7.21	11.9±8.17	
Group B	23.64±8.63	21.79±8.46	<0.01
Group C	36.86±15.11	37.4±15.75	<0.001
Group D	66.15±38.64	64.13±33.98	<0.001

P*: Serum EGFR and urinary EGFR vs. control group

EGFR was determined according to the method described by Manneck et al. in 1993[15]. Briefly, ELISA allows the quantitative determination of human EGF-R (hEGF-R). The hEGF-R in the samples is bound to an available excess of monoclonal antibodies against hEGF-R which have been mobilized on the surface of microtitre plates. After a washing step, to remove all foreign substances, a rabbit anti hEGF-R antibody carries out the quantification of bound hEGF-R. Detection of the rabbit antibody is performed by a peroxidase labeled goat antirabbit antibody (Po-antibody). The amount of converted substrate is directly proportional to the amount of bound hEGF-R and can be determined photometrically at 450nm (supplied by Immunotic, France). A standard curve was constructed by plotting the mean absorption obtained from each reference standard against its concentration in fmol/ml with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis. The average absorbance values for each set of reference standards, controls and patient samples were calculated. Using the mean absorbance value for each sample, we determined the corresponding concentration of EGFR (fmol/ml) from the standard curve (EGFR concentration [fmol/mg protein] = result in fmol/ml/protein concentration in mg/dl).

All data were expressed as mean ± standard deviation. Student (t) test, analysis of variance and correlation coefficient were used for statistical analysis. A *ROC* (receiver operating characteristic) curve was used to determine the best cut-off point to differentiate between malignant and normal urothelium. The *ROC* curve is a plot of the sensitivity on the Y-axis versus 100-specificity on the X-axis. The best cut-off point is the nearest one to the upper left corner which has the largest area under its curve.

RESULTS

A total of 40 patients were involved in the present study. Thirty patients had newly diagnosed bladder cancer (26 males and 4 females) and 10 were healthy volunteers (6 males and 4 females). The patients' age ranged from 42 to 76 years. Bilharziasis was recorded in 33 patients, 6 of them were in the control group. There was no significant difference in the serum and urinary EGFR expression between bilharzial patients and non-bilharzial patients. The mean values of the serum EGFR of bilharzial and non-bilharzial patients were 46.47±32.23 and 36±38.22 fmol/ml, respectively. The mean values of the urinary EGFR of bilharzial and non-bilharzial patients were 45.42±29.78 and 41±31.16 fmol/ml, respectively.

None of the patients with grade I bladder cancer was found to have stage T3 or T4 cancer, while all patients with grade II and III cancer had invasive cancer (>Ta) (Table 1). The urine and the serum levels of EGFR in the control group ranged from 4 – 24 and 2.5 – 26 fmol, respectively, and the mean values were 12.37±7.21 and 11.9±8.17 fmol, respectively. The urine and the serum levels of EGFR in the cancer patients ranged between 12 – 165 and 10 –142 fmol, respectively, while the mean values were 46.47±32.23 and 45.4±29.78 fmol, respectively. There was a significant increase in the serum and urinary EGFR levels in the cancer patients as compared to the control group (Table 2). In addition, a stepwise increase in serum and urinary EGFR with increasing cancer grade could be noticed. The mean values of serum and urinary EGFR showed a significant increase in all stages of bladder cancer as compared to the control group (only EGFR expression in the urine of the patients of stage Ta was not significantly

Table 3: Statistical Comparison of EGFR Expression in Serum and Urine Between the Control Patients and Different Bladder Cancer Stages

Stages	No. of Patients	Serum EGFR	Urinary EGFR	P*	P**
Control	10	12.37±7.21	11.9±8.17		
Stage Ta	2	27.5±13.44	23.5±12.02	< 0.05	> 0.05
Stage T1	7	35.59±15.19	35.07±15.1	< 0.01	< 0.001
Stage T2	12	48.08±23.45	46.83±22.15	< 0.001	< 0.001
Stage T3	6	70.83±57.09	67.67±51.0	< 0.01	< 0.05
Stage T4	3	29.33±9.71	34.0±18.74	< 0.01	< 0.01

P*: Serum EGFR vs. control group; P**: Urinary EGFR vs. control group

Table 4: Evaluation of the Serum EGFR Cut-Off Point 22 fmol and the Urinary EGFR Cut-Off Point 25 fmol in the Detection of Malignancy

	fmol	Sensitivity	Specificity	+ve Predictive Value	-ve Predictive Value	Accuracy
Serum EGFR	<22	83.3%	90%	96.2%	64.3%	85.0%
Urinary EGFR	<25	80.0%	90%	96.0%	60.0%	82.5%

different from the control group) (Table 3). In terms of tumors stages, the EGFR expression in both serum and urine showed a stepwise increase from stage Ta – T3. However, the expression of EGFR in the serum and urine of patients with stage T4 was decreased as compared to stage T2 and T3 (Table 3).

The best cut-off values for serum and urinary EGFR were 22 fmol/ml and 25 fmol/ml, respectively. The best serum cut-off point had a sensitivity of 83.3%, a specificity of 90% and an accuracy of 85%, which means that only 5 out of 30 patients would be missed in the diagnosis using this cut-off point. The best urinary cut-off point had a sensitivity of 80%, a specificity of 90% and an accuracy of 82.5%, which means that 6 out of 30 patients would have missed in the diagnosis using this cut-off point (Table 4). There was a strong correlation between serum EGFR and urinary EGFR (r=0.98) and between the stages and grades of bladder cancer (r=0.57). In addition, there was a significant correlation between tumor grades versus serum EGFR and urinary EGFR (r=0.63 and 0.67, respectively). However, the correlation matrix between serum EGFR and urinary EGFR versus tumor stages was not significant (r=0.14 and 0.21, respectively.

DISCUSSION

Bladder cancer is a major national health problem in Egypt. The incidence of urinary tumors has been reported to represent 27.11% of all malignancies in men, with bladder cancer constituting 26.39%, while all other types of malignancies in the urinary system constitute 0.27%[16]. The recurrence rate of bladder cancer is high. More than 50-70% of superficial tumors recur within 5 years and almost 90% recur within 15 years, while 10-20% progress to invasive cancer[17]. Successful management depends on regular follow-up and early detection of persistent or recurrent carcinoma. An intensive search for new urinary tumor markers has developed during the past few years. Antigens, such as P53 and M344 have been described. Urinary biomarkers such as the bladder tumor antigen (BTA), nuclear matrix protein (NMP22), CYFRA 21-1, fibrinogen and

fibrinogen degradation product have been approved for clinical use.

EGFR is a member of the C-erb B family of growth factor receptors possessing an intrinsic protein tyrosine kinase activity. There are several evidences that implicate the enhanced activity of this growth factor receptor with the progression of bilharzial and non-bilharzial bladder cancers. By means of an immunohistochemical study, Ramchurren et al.[18] detected EGFR in 76% of bilharzial bladder cancer. In addition, flow cytometric analysis of paraffin-embedded material showed an increased EGFR expression in aneuploid bladder cancer. In the present study, the EGFR level was determined by the ELISA assay. This method is very practical because it does not require sophisticated equipment, can be performed within a short time and has no hazards of irritation. There is no contrast between the results of our study by the ELISA technique and the results of radioligand binding as reported by Harney et al.[3] or the immunohistochemistry method reported by Ravery et al.[19] for EGFR determination in bladder cancer patients.

Clinical and urinary tract mapping studies suggest that transitional cell carcinoma is usually a field change disease with tumors arising at different times and sites in the urothelium (polychronotypical disease). An abnormal distribution of EGFR has been observed in the dysplastic and normal appearing urothelium, both near-by and remote from transitional cell cancer[7]. Different authors emphasized that urinary EGF/urothelial EGFR interaction is actually important in urothelial tumorigenesis. Moreover, they suggested that urinary EGF/urothelial EGFR interaction might encourage malignant cell motility and angiogenesis as well as proliferation and all processes important for invasion[2,7]. Since the major ligand for these receptors, EGF, is excreted in high concentration in biologically active forms, an abnormally high expression of EGFR may be an example of a cell taking advantage of its unique environment to provide it with growth advantage[20]. Evaluation of the EGFR in the urine has not been tried before. Previous studies looked at EGF levels in the urine of patients with bladder cancer but did not look for its receptors[20,21].

In the present study we found that EGFR increased significantly in the urine of patients with a bladder tumor. In addition, there was a stepwise increase in the urinary EGFR expression with increasing tumor grades. Furthermore, there was a strong correlation between tumor grades and urinary EGFR (r=0.56). Under normal circumstances, EGFR is confined to the basal layer of the epithelial cells. In transitional cancer, however, EGFR becomes expressed on cells of all layers including those of luminal surfaces. EGFR reaches the urine due to shedding of the extra cellular domain of receptors present in the surface of the tumor cells in which extreme urinary acidification may help rapid dissociation of EGF, EGFR or ligand-receptor complex[22].

A significant increase in the serum EGFR in bladder cancer patients was observed in the present study. In addition, there was a significant increase in the serum EGFR with increasing tumor grade. In agreement with our results, both Messing[7] and Neal[13] found that the degree of expression of EGFR in bladder cancer directly correlates with the invasive phenotype. The increased level of EGFR and the aggressive biological behaviour of bladder cancer have been found in different studies. Kiyokawa et al. studied the relation between EGFR and the cell cycle and reported that EGFR did not respond to ligand stimulation in the M phase; they suggested that a negative regulation of the ligand-receptor interactions in the M phase might control the normal function of the receptors and that EGFR over expression in bladder cancer will disrupt this cycle-dependent regulation of the receptors[23]. On the other hand, Mellon et al. reported no significant correlation between serum EGFR level and tumor grades and stages of bladder cancer patients[24]. They explained this finding to be due to the fact that most of their patients had a relatively high rate of tumor proliferation.

In the present study the mean value of serum and urinary EGFR showed a stepwise increase from stage Ta up to stage T3; however, the EGFR expression declined in stage T4 (Table 3). This result explains why the correlation matrix between serum EGFR and urinary EGFR versus tumor stages was not significant (r=0.14 and 0.21, respectively). Ravery et al.[1] reported the same results. They found that EGFR expression increased in invasive bladder cancer but declined in the infiltrating tumor (T4). The reason for the heterogeneity of receptor distribution in the infiltrating tumor is due to a suppression of the active division of the basal cells which occurs in the superficial tumors leading to a loss of cell-cell cohesions and interactions.

In the present study there was no significant difference between urinary EGFR expression in Ta patients and the control group. This might be a positive finding pointing to the possibility that increasing EGFR expression occurs in invasive cancer only, or the data might just be attributed to the small number of patients (only 2 patients) in this group.

We tried to evaluate the efficacy of EGFR as a biological marker for the prediction of malignant disease by detecting the best cut-off point (in serum and urine) that can differentiate between normal and malignant urothelium using the *ROC* curve. The best cut-off values for serum and urinary EGFR were 22 fmol/ml and 25 fmol/ml, respectively. The best serum cut-off point had a sensitivity of 83.3%, a specificity of 90% and an accuracy of 82.5%. The best urinary cut-off point showed a sensitivity of 80%, a specificity of 90% and an accuracy of 82.%. In fact, no cancer patient had a serum or urine EGFR of less than 10 fmol/ml, however using 10 fmol/ml as a cut-off point would decrease the specificity to 40% and 50% in serum and urine, respectively. Nevertheless, the use of these cut-off points in screening for bladder cancer in high-risk patients to detect the recurrence of superficial tumors needs further evaluation.

In conclusion, the results of the present study suggest that the high levels of EGFR in the serum and in the urine of patients with bladder cancer are related to various malignant histological features (tumor grade) and invasiveness of bladder cancer and, particularly, to cell proliferation. This test can be used as an adjunct to histological grading and other biological tumor markers presently available, such as P53 and BTA in determining the aggressiveness of the bladder tumor. In the future, the role of EGFR should be evaluated for the prediction of the recurrence rate, the response to systemic treatment and for screening for bladder tumors in the early stage when radical treatment is still a valid option. In addition, the possibility of using anti-EGFR monoclonal antibodies as therapeutic agents which act as EGF antagonists may have a potential use in chemotherapy by blocking EGF-related peptides in the tumor with over expression of EGFR.

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