

Evaluating the Prevalence of the Epidermal Growth Factor Receptor in Transitional Cell Carcinoma of Bladder and its Relationship With Other Prognostic Factors

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Abstract

Background: The most common malignancy in the urinary system has been bladder cancer and the most predominant histologic subtype has been transitional cell carcinoma (TCC). There were many molecular risk factors, related with poor prognosis. One of these factors was expression of epidermal growth factor receptor (EGFR).

Objectives: The aim of this study was to evaluate the prevalence of the epidermal growth factor receptor in transitional cell carcinoma of bladder and its relationship with other prognostic factors.

Patients and Methods: This analytic descriptive study has performed with 61 patients with TCC of bladder after radical cystectomy whom have been hospitalized in Labbafinejad hospital in Tehran, Iran between 2007 and 2010. We have used Chi-square and t-test to analyze our data samples.

Results: Records of 61 patients have studied. Fifty three of the total samples were positive for EGFR expression (86.9%). Fifty samples of these fifty-three belonged to men and three others were women's samples ($P = 0.46$). Among the group with EGFR expression the results were as follows: 25 patients (47.2%) were 60 years old or less and 28 patients (52.8%) were older than 60 ($P = 0.023$), 16 patients (30.2%) had invasion to lamina propria, and the rest of them had invasion to deeper layers ($P = 0.56$). For most patients we could not determine the invasion of tumoral cells into the lymph nodes (Nx) ($P = 0.067$). Thirty four patients (64.2%) had not lymphovascular invasion ($P = 0.44$) and in forty three of patients (81.1%), perineural invasion have not seen ($P = 0.23$). Finally, 36 patients (67.9%) were grade 3 ($P = 0.27$).

Conclusions: In this study we have concluded that most patients had EGFR positive expression. Also, except for the age, there was not any significant relation between expression of EGFR and the other prognostic factors such as, gender, invasion of the tumor into the layers, involving the lymph nodes, lymphovascular or perineural invasion, and grading.

Keywords: Epidermal Growth Factor Receptor, Prognostic Factors, Transitional Cell Carcinoma

1. Background

The most common malignancy in the urinary system has been bladder cancer (1) that has suggested that this malignancy was a main cause of cancer morbidity or mortality (2). Bladder cancer has been the ninth most common cancer in all over the world (1). In western countries, the most predominant histologic subtype in urothelial system has been transitional cell carcinoma (TCC) (urothelial carcinoma) (3). Genetic abnormalities and environmental exposures were responsible for most causes of this malignancy (4). Overexpression of some growth factors, such as epidermal growth factor receptor (EGFR), has mentioned in many tumors like gliomas, pancreatic, colorectal, breast, head and neck, lung, prostate, ovary, advanced gastric cancer, and urothelial carcinoma (5-7). In many contexts, it has reported that EGFR

family encoded by oncogenes c-erb. These receptors have belonged to the group of receptors that all have encoded by these oncogenes. Human epidermal receptors (HER), family of tyrosine kinases receptors were four different receptors. Loss of coordination for cell growing by pathological expression of c-erbB oncogenes, could lead to malignancies. Motility, angiogenesis, invasion and metastasis all together were the results of EGFR expression (8-11). The higher stage or grade of bladder cancer has expected with more expression of EGFR, thus eventually progression of carcinoma has caused poor prognosis (12-19). Also for anti-tumor effects, one could inhibit these pathways. Inhibition of EGFR1 and HER2 might have effective results for their antitumor activity because numerous bladder tumors expressed the EGFR family (20).

2. Objectives

The aim of this article was evaluating the prevalence of the EGFR in transitional cell carcinoma of bladder, and its relationship with other prognostic factors such as: age, gender, invasion of the tumor into the layers, involving the lymph nodes, lymphovascular or perineural invasion, and grading.

3. Patients and Methods

3.1. Research Design and Setting

This descriptive-analytic study has performed with sixty one patients with TCC of bladder according to their biopsies whom have been hospitalized in Labbafinejad hospital in Tehran, Iran between 2007 and 2010.

3.2. Sampling

The sample size has calculated 61, using the following formula:

$$(1) \quad n = \frac{Z^2 p(1-p)}{d^2}$$

Formula character means: n = sample size; Z = the appropriate value from the normal distribution for 95% confidence (1.96); p = the anticipated prevalence; d = confidence interval.

3.3. Measurement Tool

Patients have referred to the urology department from 2007 to 2010, have evaluated carefully and anyone who have treated with radical cystectomy and had TCC of bladder, have selected for further investigation. Their pathological blockshave stained with antibody (Dako, Denmark) for evaluating the presence or absence of EGFR. EGFR expression has considered positive if have evaluated in more than 10% of the stained samples, otherwise it has considered negative.

3.4. Data Collection

As mentioned, this study has performed with 61 patients with TCC of bladder according to their pathologic results after radical cystectomy, whom have been hospitalized in Labbafi Nejad hospital in Tehran, Iran between 2007 and 2010. Samples have evaluated by immunohistochemistry (IHC) study for EGFR expression, then relationship with other prognostic factors like age, gender, grade, lymphovascular or perineural invasion, and finally TNM staging studies.

3.5. Ethical Consideration

Consent has prepared for each patient. Case patients have been anonymous, then researchers have allowed to use information of the records. Method of this study was completely harmless and noninvasive. Ethical approval

for the inquiry has obtained from the ethical review group and The deputy of research at the Shahid Beheshti University of Medical Sciences under code 74.

3.6. Statistical Analyses

We have used Chi-square and t-test for data analyzing. The statistical analyses have performed by using software SPSS version 17. P value for statistical significance has defined as $P < 0.05$.

4. Results

Sixty one patients have included in the research, 57 samples (93.4%) were men, and 4 others (6.5%) were women. The average age of the patients has determined as 64 ± 19 years. The average age for men was 64.2, and for women was 63.2 years. Fifty-three of samples were positive for EGFR expression (86.9%), and 8 specimens were negative (13.1%). Among positive results for expression of EGFR, 50 specimens have belonged to men (94.3%), and three samples pertain to women (5.7%). There was not a significant relation between gender and expression of EGFR ($P = 0.46$). In group with expression of EGFR, 28 (52.8%) patients were older than 60 and 25 (47.2%) patients were 60 or younger. There was a significant relation between age and expression of EGFR ($P = 0.023$). In specimens with positive results for EGFR, invasion to bladder wall was as follows: invasion to lamina propria has seen in 16 patients (30.2%), invasion to superficial muscles has found in 12 patients (22.6%), invasion to deep muscular layer has seen between 9 patients (17%), invasion to fat tissues around the bladder have observed in 11 patients (20.8%), and involvement of adjacent organs have observed in 5 patients (9.4%). We have excluded the noninvasive TCC samples from the study. There was no significant relationship between expression of EGFR and invasion thickness ($P = 0.56$). Among positive results for EGFR, invasion to regional lymph nodes in 40 patients (75.5%) was unknown, and in 11 patients (20.8%) there was no regional lymph node involvement.

In two patients (3.8%) there was one positive pelvic lymph node (N1), and nobody had multiple pelvic lymph node involvement (N2). Thus, there was no significant meaning between EGFR expression and pelvic lymph node involvement ($P = 0.06$). Among the group with positive results according to EGFR expression, distant metastasis in 51 (96.2%) of patients were unknown, but in one patient (1.9%) distant metastasis has detected, and the other one had not any metastasis. So, we could not determine any connection between EGFR expression and distant metastasis ($P = 0.85$) and we have excluded them from the study. In the group of specimens with this receptor, lymphovascular invasion was positive in 19 patients (35.8%) and 34 patients (64.2%) had not this type of invasion and there was no significant relationship ($P = 0.44$). In positive EGFR expression samples, perineural invasion was positive in 10 patients (18.9%) and 43 patients had not perineural invasion. Thus no significant relationship has found ($P = 0.23$). Finally, in pa-

tients with EGFR expression, the grading of the tumor was as follows: low grade (G1) in 4 patients (7.5%), intermediate grade (G2) in 13 patients (24.5%), and high grade (G3) in 36 patients (67.9%) has seen, and there was not any meaningful relationship among EGFR expression and tumor grading ($P = 0.27$) (Table 1).

Table 1. EGFR Expression and the Other Prognostic Factors in 63 Patients With TCC^a

Prognostic Factor	EGFR Positive	EGFR Negative	P Value
Age, y	53 (100)	8 (100)	0.023
≤ 60	25 (47.2)	2 (28.6)	
> 60	28 (52.8)	5 (71.4)	
Gender	53 (100)	8 (100)	0.466
Female	3 (5.7)	1 (12.5)	
Male	50 (94.3)	7 (87.5)	
Invasion	53 (100)	8 (100)	0.569
Lamina Propria	16 (30.2)	3 (37.5)	
Superficial Muscle	12 (22.6)	0 (0)	
Deep Muscle	9 (17)	1 (12.5)	
Perivesical Tissue	11 (20.8)	3 (37.5)	
Other Organs	5 (9.4)	1 (12.5)	
Primary Tumor	53 (100)	8 (100)	0.48
T1	17 (32.1)	3 (27.5)	
T2	20 (37.7)	1 (12.5)	
T3	10 (18.9)	2 (25)	
T4	6 (11.3)	2 (25)	
Regional Lymph Nodes	53 (100)	8 (100)	0.067
N0	11 (20.8)	2 (25)	
N1	2 (3.8)	0 (0)	
N2	0 (0)	1 (12.5)	
NX	40 (75.5)	5 (62.5)	
Distant Metastasis	53 (100)	8 (100)	0.856
M0	1 (1.9)	0 (0)	
M1	1 (1.9)	0 (0)	
MX	51 (96.2)	8 (100)	
Grade	53 (100)	8 (100)	0.27
G1	4 (7.5)	2 (25)	
G2	13 (24.5)	1 (12.5)	
G3	36 (67.9)	5 (62.5)	
LVSI	53 (100)	8 (100)	0.441
Positive	19 (35.8)	4 (50)	
Negative	34 (64.2)	4 (50)	
PNI	53 (100)	8 (100)	0.23
Positive	10 (18.9)	3 (27.5)	
Negative	43 (81.1)	5 (62.5)	

Abbreviations: EBRT, external beam radiotherapy; LVI, lymph-vascular space invasion; PNI, perineural invasion; TCC, transitional cell carcinoma.

^aValues are expressed as No.(%).

5. Discussion

Based on our findings most of patients (86.9%) with TCC of bladder, expressed the EGFR and only eight patients (13.1%) had negative expression. Also, except for the age, there was not any significant statistical relationship between expression of EGFR and other prognostic factors such as gender, invasion of the tumor, involvement of pelvic lymph nodes, lymphovascular invasion, perineural invasion, and grading of the tumor. For distant metastases, our information was not definitive. Likewise our findings, results of a study have aimed to evaluate the relationship between over expression of EGFR and other prognostic factors, shown that apart of tumor staging, expression of this receptor in TCC of bladder had not any role in prognosis and there was not any relationship between expression of this receptor and other prognostic factors (19). Another study has shown between 52% of patients, EGFR was present, but there was an inverse relation between this presence and the degree of tumor invasion or tumor grade, also, survival was better in this group ($P = 0.001$) (21). Another report about high grade tumors has mentioned an inverse relationship between tumor invasion and EGFR expression (22). In another report the authors have concluded that over expression of EGFR has correlated with improved disease specific survival (23). Results of another study have indicated that over expression of the EGFR apart of disease stage could not be an independent predictive factor for survival (19). On the other hand, in one study it has shown that there was a meaningful relationship between stage of the cancer and EGFR expression (22). Over expression of EGFR has shown as an independent predictor factor for survival according to results of two multivariate analyses (18, 19, 24). In a Meta analyses that has collected the results of more than 200 studies with nearly 20000 patients who suffered from several cancer types, the relationship between survival and expression of EGFR has demonstrated (25).

In another multivariate analyses, an important correlation between survival of patients and expression of EGFR has declared (26). But EGFR expression as a predictor factor of survival has not defined to have an independent role apart from tumor stage. In one study, 5 years survival was 41% in patients with EGFR expression, on the other hand, the results for negative expression were 60%. Another point is that EGFR expression has related with disease specific mortality (19). As mentioned in previous study about 121 patients with TCC of bladder who have treated with radical cystectomy with curative purpose, 5 years cancer specific survival in forty seven patients with mild or moderate expression of EGFR was 60% and this estimation for high EGFR expression among 45 patients was 41% ($P = 0.03$) (19). In an inquiry that has done on 110 blocks of tumor specimen after bladder surgery and radiotherapy, 72% of samples were positive for EGFR expression. Good responses to radiotherapy had a meaningful correlation with specimens that had not express EGFR and these patients had grater life expectancy. As a result,

suppression of these receptors probably might lead to produce good responses and that was the hypothesis (27). There were two main results of this study:

First one: The majority of patients (86.9%) with TCC of bladder expressed the EGFR; second one: except for the age, there was no significant statistical correlation between expression of EGFR and other prognostic factors such as, gender, tumor invasion into layers, involvement of pelvic lymph nodes, lymphovascular or perineural invasion, and grading. Also, data for distant metastases, were inconclusive. One reason which might explain the conclusion could be the low number of patients, other reason could be incomplete information of patients' records such as the numbers of pelvic lymph node involvement or distant metastasis. For better finding about association between EGFR expression and other prognostic factors, research needs more patient samples and complete information from each patient.

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Footnotes

Authors' Contribution: Mahmoud Parvin and Parto Sabet-Rasekh are joint authors. All authors have participated for this paper and reviewed the final manuscript. Parisa Sabet-Rasekh and Parto Sabet-Rasekh have performed, analyzed and drafted data collection. Mahmoud Parvin and Peyman Mohammadi Torbati, have stained and evaluated all blocks. Hamidreza Mirzaei has participated in the study conception, revised and coordinated it for drafting manuscript and was corresponded author of the paper. Parastoo Hajian has revised and helped to draft the article.

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