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Pathology

EGFR EXPRESSION IN PRIMARY EPITHELIAL URINARY BLADDER TUMORS AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

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ABSTRACT

Introduction: EGFR is over expressed in many epithelial tumors including urothelial carcinomas. Over expression of EGFR is considered as a poor prognostic marker in various studies. Thus, the present study was done to evaluate the EGFR expression in primary urothelial carcinomas and its correlation with clinicopathological parameters.

Methods: This was a cross-sectional, observational study carried out between June 1, 2019 to May 31, 2020 in the Department of Pathology and Urology, Indira Gandhi Medical College, Shimla. One hundred and ninety seven patients with primary epithelial urinary bladder cancer were included. Patients with inflammatory and metastatic lesions of urinary bladder and post chemoradiotherapy were excluded. The correlation between EGFR expression and the various factors like age (<60 years or ≥60 years), sex (male/female), size of tumor (< 3cm or ≥ 3cm), number of tumors (solitary/multiple) and grade (high/low) were evaluated using EpiInfoV.7 software version and chi-square test.

Results: The age of patients ranged from 36 to 89 years. Male preponderance was observed. Most common clinical presentation was painless hematuria. EGFR positivity was observed in majority, 190 cases, irrespective of histological type and grade of tumor. No statistical significant correlation was found between EGFR expression and age of patient, size of tumor, number of tumor and histological grades of urothelial tumors.

Conclusion: Majority of urothelial carcinomas over expressed EGFR irrespective of their histological grade. So, targeted EGFR therapy in urothelial carcinomas can emerge as a novel therapy improving overall survival and prognosis of the patients with urothelial carcinomas.

KEYWORDS : EGFR, Urothelial carcinoma

INTRODUCTION

Urinary bladder lesions are responsible for significant morbidity and mortality throughout the world. Bladder cancer has become very common with almost 5,50,000 new cases reported worldwide in 2018. It is the 6th most commonly occurring cancer in men and ranks 17th in women [1]. In India, the incidence of urinary bladder cancer has been reported as 3.67% among males and 0.83% in females with overall incidence being 2.25% [2].

Majority of bladder tumors are epithelial in origin. Most common are urothelial/transitional neoplasms comprising approximately 90% of all primary tumors followed by squamous cell carcinoma (5%) and primary adenocarcinoma (2%). Small cell carcinoma and sarcomas are encountered much less frequently [3].

Most cases of urothelial carcinoma present in patients between 50 -80 years of age group with male to female ratio of 3 to 4:1 [3].Cigarette smoking, occupational carcinogens from chemical industry, Schistosoma hematobium infection in endemic areas, use of artificial sweeteners are known risk factors [4]. Most common presenting symptom is painless hematuria. Others symptoms may be dysuria, urgency, frequency, palpable pelvic mass, weight loss etc [5].

Cystoscopy is the primary diagnostic modality for patients with suspected bladder tumors used for localization and performing biopsy of suspected lesions. Trans urethral removal of bladder tumor (TURBT) is a therapeutic procedure that allows assessment of degree of differentiation and depth of tumor invasion which are important parameters for diagnosis and prognosis. Tumor size, stage, grade and multifocality are important factors predictive of tumor progression [4].

The natural history of bladder cancer is dependent on the stage and grade of the initial tumor. Patients with non-muscle invasive bladder cancer (NMIBC) have good prognosis. They are treated with TURBT followed by intravesical BCG or Mitomycin C.

Muscle invasive bladder cancer (MIBC) is more aggressive and is treated by radical cystectomy with or without chemotherapy. Systemic chemotherapy is used as the first line of treatment in advanced stages of bladder cancer[6].

Given the poor prognosis of MIBC much work has been undertaken to determine clinical and molecular prognostic markers predictive of progression in NMIBC [7-10]. One such factor is epidermal growth factor receptor (EGFR). EGFR is over expressed in many epithelial tumors including non-small cell lung cancer, colorectal, gastric, pancreatic, ovarian and breast cancers [11]. Over expression of EGFR in bladder cancer has been widely reported and several studies have shown EGFR positivity to be associated with high tumor stage, tumor progression, and poor clinical outcome making it a potential therapeutic target [11,12,13] There are several methods of inhibiting the activity of EGFR including use of tyrosine kinase inhibitors, monoclonal antibodies against EGFR, immunotoxin conjugates, and antisense oligonucleotides [14]. Use of an anti-EGFR therapy, in combination with more conventional treatment may improve the response to treatment and overall survival of the patients with urinary bladder cancer.

Thus, the present study evaluated the EGFR expression in primary urothelial carcinomas and its correlation with clinicopathological parameters.

METHODS

This was a cross-sectional, observational study carried out between June 1, 2019 to May 31, 2020 for a period of one year in the Department of Pathology and Urology, Indira Gandhi Medical College, Shimla. The study protocol was approved by Himachal Pradesh University Ethics Comittee. To comply with the ethical principles, informed consent was obtained from each study participant. One hundred and ninety seven patients (TURBT and radical cystectomy) with primary epithelial epithelial urinary bladder cancer were included in the study. Patients with inflammatory and metastatic lesions of urinary bladder and post chemoradiotherapy were excluded from the study. Clinical presentation and cystoscopic findings (size, number and shape of tumor) of these patients were noted. All samples were fixed in 10% neutral buffered formalin and paraffin embedded. Diagnosis was made as per WHO/ISUP classification (2004) [15]. Urothelial tumors were graded into low grade and high grade categories and their invasion into lamina propria and muscularis propria was assessed according to WHO/ISUP (2004) classification. Immunohistochemistry for EGFR expression was

done on all 197 cases using rabbit monoclonal anti-EGFR antibody with standard IHC staining protocol. Positive and negative controls were run simultaneously with all specimens. Sections from paraffin blocks of already diagnosed cases of non-small cell lung carcinoma with known positivity for EGFR were used as positive control. Phosphate buffer was used instead of primary antibody in negative control.

Samples were evaluated at high magnification, intensity of staining as well as proportion of stained cells were scored and a composite score was obtained [16]. The staining intensity was scored on a semi-quantitative 4-point scale: 0-equivalent to the negative control, 1-weak cytoplasmic stain slightly darker than negative control, 2-moderate stain (defined as an intensity of score 1-3), 3- intense stain equivalent to or darker than the positive control. The percentage of stained cells was also scored on a semi-quantitative 4-point scale as: 0 for no positive cells, 1- 0 to 25% stained cells, 2- 26 to 50% stained cells, 3- 51 to 75% stained cells and 4- >75% stained cells. Final scoring was then based on composite score obtained by multiplying the score of staining intensity and percentage of stained cells: a score of 0 was negative, 1-4 was + (weak), 5-8 was ++ (moderate), 9-12 was +++ (strong).

The correlation between EGFR expression and the various factors like age (<60 years or ≥60 years), sex (male/female), size of tumor (< 3cm or ≥ 3cm), number of tumors (solitary/multiple) and grade (high/low) were evaluated. The statistical significance of EGFR expression was calculated using EpiInfoV.7 software version and chi-square test was applied. Significance was assumed at a p value less than 0.05.

RESULTS

One hundred ninety seven specimens including both TURBT (191) and cystectomy (6) were received in the Department of Pathology. The age of patients ranged from 36 to 89 years with mean age of 62 years. Male preponderance was observed in this study with male to female ratio of 6.9:1. Majority, 141 (71.6%) patients, were smokers and 113 (57.4%) patients had agricultural background. Most common clinical presentation was painless hematuria found in 173 (87.8%) patients followed by lower abdominal pain (5.6%), urinary flow obstruction (4.6%) and dysuria (2%). Solitary tumors were present in 134 (68%) patients and in 111 (56.3%) patients tumor was <3 cm in size. Ninety-nine (50.2%) patients had papillary mass.

Most common histological type found was urothelial carcinoma (98%) followed by other types such as squamous cell carcinoma (1%), small cell carcinoma (0.5%) and adenocarcinoma (0.5%). Among urothelial tumors (193), majority, 126 (65.3%) cases, were high-grade urothelial carcinoma followed by 67(34.7%) cases of low-grade urothelial carcinoma. Among HGUC, 99 cases had papillary configuration and 27 were non-papillary invasive carcinomas and its variants.(Table 1).

Table 1. Histomorphological Spectrum Of Urinary Bladder Tumors According To WHO 2004 Classification

S. No.	Histological type of tumor	No. of cases	Percentage
1.	Urothelial carcinoma-high grade	126	64%
1.1	Papillary	99	
1.2	Non - papillary invasive	27	
	Invasive urothelial	16	
	Squamous differentiation	4	
	Glandular differentiation	2	
	Sarcomatoid	1	
	Clear cell	2	
	Plasmacytoid	2	
2.	Urothelial carcinoma-low grade	67	34%
3.	Squamous cell carcinoma	2	1%
4.	Small cell carcinoma	1	0.5%
5.	Adenocarcinoma	1	0.5%
	Total	197	100%

Out of 67 cases of low grade urothelial carcionoma (LGUC), in 11 cases no muscle tissue was included in the specimen. Hence, invasion was assessed in 56 cases. Forty-six (82.1%) cases were non-invasive while in 10 (17.9%) cases invasion was limited up to lamina propria. None was muscle invasive (Table 2). Out of 11 cases (muscle tissue not included), 10 (90.9%) cases were non- invasive while in 1 (9.1%) case there was lamina propria invasion (Table3).

Out of 126 cases of high grade urothelial carcinoma (HGUC), in 4 cases no muscle tissue was included in the specimen. Hence, invasion was assessed in 122 cases. Majority, 95 (77.9%) cases, showed both lamina propria and muscle invasion. Invasion was limited up to lamina propria in 24(19.7%) cases while 3(2.4%) cases were non-invasive (Table 2). In all 4 cases (muscle tissue not included), lamina propria invasion was present (Table 3).

Table 2. Invasion In Different Histological Grades Of Urothelial Tumors

Grade of tumor	Non-invasive	Invasive		Total cases
		Invasion up to lamina propria	Invasion into lamina propria and muscle	
LGUC	46(82.1%)	10(17.9%)	-	56
HGUC	3(2.4%)	24(19.7%)	95(77.9%)	122

Table 3. Invasion Assessment Of Specimens In Which Muscle Tissue Was Not Included

Grade of tumor	Non-invasive	Lamina propria invasion	Total cases
LGUC	10(90.9%)	1(9.1%)	11
HGUC	None	4(100%)	4

EGFR expression was evaluated in all 197 cases of urinary bladder tumors and scored immunohistochemically. Urothelial cells show EGFR positivity as brownish cytoplasmic and/or membranous staining. In the present study, EGFR positivity was found on the cell membrane and in the cytoplasm. EGFR expression showed intra-tumoral variation. Invasive carcinoma cells were usually positive for EGFR. In weakly staining cases basal cells were often positive for EGFR.

Majority, 190 cases, showed EGFR positivity irrespective of histological type and grade of the tumor. Out of 193 cases of urothelial carcinoma, majority, positive EGFR expression was seen in 187 cases. Two cases of squamous cell carcinoma and one case of adenocarcinoma revealed strong immunoreactivity while one case of small cell carcinoma showed negative EGFR expression. Among 67 cases of LGUC, 8 cases showed weak EGFR expression while moderate and strong expression was observed in 18 and 39 cases respectively (Figure1). Only 2 cases were negative for EGFR expression. In HGUC, 6 cases showed weak EGFR expression while moderate and strong expression was found in 16 and 100 cases respectively (Figure2). However, 4 cases were negative for EGFR expression (Table 4).

Table 4. EGFR Expression In Different Histological Types Of Urinary Bladder Tumors

Histological diagnosis	Number of cases	EGFR Expression	
		Positive	Negative
Urothelial carcinoma	193	187	6
Squamous cell carcinoma	2	2	-
Adenocarcinoma	1	1	-
Small cell carcinoma	1	-	1

We did not observe any statistical significant correlation of EGFR expression with age of patient, size of tumor, number of tumor and histological grades of urothelial tumors. EGFR expression correlated significantly with sex of the patients(Table 5 and 6).

Table 5. Association Of EGFR Expression With Clinical Parameters

Variable	Number of patients	EGFR expression		P value
		Negative	Positive	
Sex				
Male	172	6	166	<0.05
Female	25	1	24	
Age				
<60 years	58	3	55	0.4538
≥60 years	139	4	135	
Number of tumor				
Solitary	111	4	107	0.9785
Multiple	86	3	83	

Size of tumor				
<3cm	134	5	129	0.8868
≥3cm	63	2	61	

Table 6. EGFR Expression (CS) In Different Histological Grades Of Urothelial Carcinomas

Histological grade	EGFR EXPRESSION (Composite Score)				Total cases	P value
	Negative	+	++	+++		
LGUC	2	8	18	39	67	0.9750
HGUC	4	6	16	100	126	

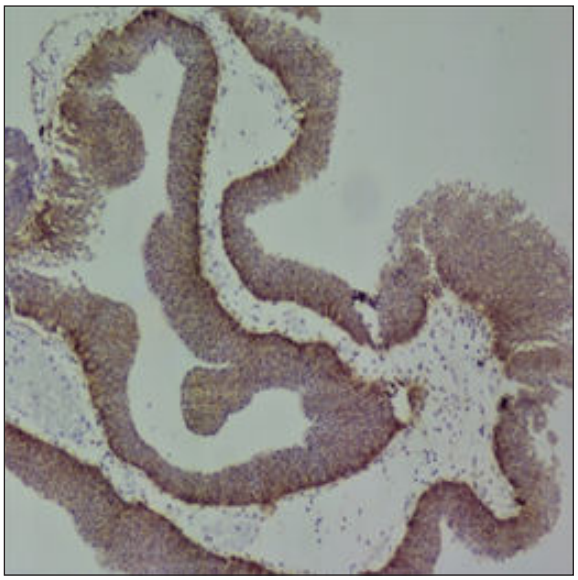

Figure1. Low grade papillary urothelial carcinoma (EGFR 3+ X 100)

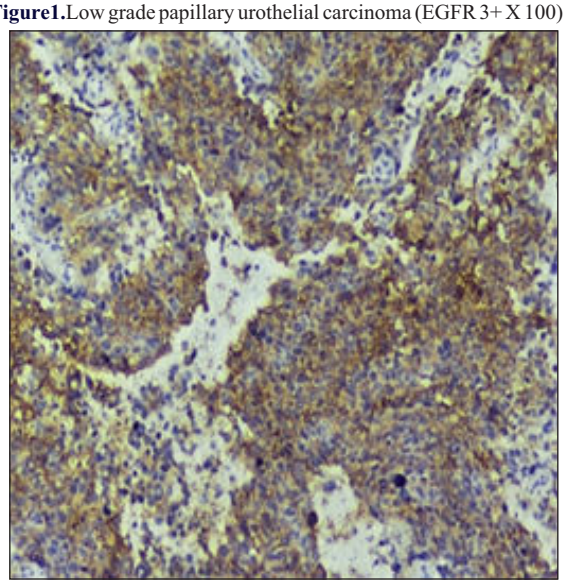

Figure2. High grade urothelial carcinoma (EGFR3+X 100)

DISCUSSION

EGFR is a 170 k Da protein that consists of three distinct structural parts: an extracellular ligand binding domain, a transmembrane domain and an intracellular tyrosine kinase domain. The extracellular component forms the ligand binding region that is activated by one of several ligands such as epidermal growth factor (EGF), transforming growth factor-alpha (TGF-α), amphiregulin etc. Activation of EGFR by one of its respective ligands leads to formation of both homodimers (with other EGFR) and heterodimers (with other members of the c-erbB family). This conformational change leads to phosphorylation of the tyrosine residues located within the autophosphorylation domain [11,12].

Phosphorylation of these tyrosine residues allows for recruitment of ATP to the catalytic kinase domain of EGFR, which allows for phosphorylation of effector molecules. Thus, a phosphorylation cascade is set off, leading to activation of various intracellular signaling pathways that have been implicated in tumorigenesis and cancer progression, including the RAS/MAPK (mitogen-activated protein kinase), PI3K (phosphoinositide 3-kinase)/AKT, and STAT3 (signal transducer and activator of transcription 3) pathways. It also results in increased angiogenesis and reduced apoptosis which promotes continuing malignant growth [11,12]. EGFR is over expressed in up to 74 per cent of bladder cancer tissue specimens but has a relatively low expression in normal urothelium. EGFR is localized to the basal layer of urothelial cells in normal urothelium but is present in both the luminal and basal layers of urothelial cells in bladder cancer. Superficial layers of normal urothelium do not express EGFR, thus protecting the basal cells from mitogenic effect of urinary EGF [17].

In the present study, EGFR was over expressed in 96.9% of urothelial carcinomas. Barua SK et al [18] observed EGFR over expression in 92.5% of cases in total of 40 cases. Chow et al [19], in their study of 245 cases, found EGFR over expression in 72.2% of the patients with urothelial carcinomas. Badawy et al [20] found 80% EGFR positivity among 30 cases of urothelial carcinomas. However, Li W et al [16] observed EGFR over expression in only 55.4% of urothelial carcinomas in total of 56 samples.

We did not find statistically significant correlation of EGFR expression with age of the patient while significantly correlation was found between EGFR expression and sex of the patient ($p<0.5$). Parvin M et al [21], in their study of 57 cases of urothelial carcinoma did not find any statistical correlation of EGFR expression with sex but found a significant relation with age. However, Sriplakich et al [22], Barua SK et al [18], Li W et al [16] found no correlation with either of parameter.

On comparison of cystoscopy findings and EGFR expression, no significant correlation of EGFR expression was observed with tumor size, number and configuration. Chow et al [19] and Barua SK et al [18] found significant correlation of EGFR expression with increase in the size of tumor. Neal et al [23] found significant association of EGFR expression with both number and size of tumor.

In our study we found EGFR over expression in both high and low grade urothelial carcinomas. No statistical significant correlation was found between EGFR over expression and histological grade of the urothelial carcinomas ($p=0.9$). Ngyuen et al [24] in their study of 85 cases did not find any significant correlation between EGFR over expression and histological grade of the tumor ($p>0.13$). Chow et al [19] also did not find any statistical significant correlation between EGFR over expression and histological grade of the tumor ($p=0.145$). Similarly, Sriplakich et al [22] and Parvin M et al [21] observed no relation between presence of EGFR over expression and histological grade. However, some of the studies have found statistically significant correlation between EGFR over expression and high grade urothelial carcinomas [16,18]. Discordance with these studies may be due to the small sample size of these studies. We had significantly more cases of LGUC, most of which revealed EGFR over expression. Discordance can also be explained due to variability in interpretation of EGFR immunostaining. Various authors have used different methods for evaluating EGFR expression.

Over expression of EGFR in urothelial carcinomas makes this receptor a good therapeutic target. Given the EGFR over expression in low grade urothelial carcinomas also, anti-EGFR therapy may emerge as a selective and novel therapy for treatment and prevention of its progression to high grade muscle invasive bladder cancer. Anti-EGFR therapy may yield good results in patients with advanced bladder cancer which do not respond to conventional treatment protocols.

Main limitation of the present study was the absence of standard method for interpreting EGFR score. Results of various studies were difficult to compare as methods of evaluating EGFR immunostaining were different. Hence, there is a need for standardization of EGFR immunostaining protocols and methods of interpretation.

REFERENCES

1. Wcrforg.Wcrforg.[online]. Available from: https://www.wcrf.org/diet and cancer-trends/bladder cancer statistics.[Accessed April 2019].
2. Naik DS, Sharma S, Ray A, Hedau S. Epidermal growth factor receptor expression in urinary bladder cancer. Indian J Urol. 2011;27:208-14.
3. Gupta A, Kaur S, Gulwani HV. Retrospective Analysis of Urinary Bladder Tumors- A study from Central India. Sch J App Med Sci. 2017;5(11E):4626-31.
4. SS, Patil GS, Patil SB. A Study on Histopathological Spectrum of Lesions in Urinary Bladder Specimens. Ann of Pathol and Lab Med. 2018;5(5):489-95.
5. Goyal B, Rao S, Sahi R, Jaiswal S. A histopathologic study of urinary bladder tumors at tertiary care center in Mid-Western region of Nepal. Asian J Med Sci. 2018

Oct29;9(6):45-50.
6. Siddiqui MR, Railkar R, Sanford T, Crooks DR, Eckhaus MA, Haines D, Choyke PL, Kobayashi H, Agarwal PK. Targeting epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) expressing bladder cancer using combination photoimmunotherapy (PIT). Sci Rep. 2019 Feb 14;9(1):1-4.
7. Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part B: Prostate and Bladder Tumours. Eur Urol. 2016 Jul;70(1):106-19
8. Ames BN, Kammen HO, Yamasaki E. Hair dyes are mutagenic: identification of a variety of mutagenic ingredients. Proc Natl Acad Sci U S A. 1975 Jun;72(6):2423-7.
9. Linn JF, Sesterhenn I, Mostofi FK, Schoenberg M. The molecular characteristics of bladder cancer in young patients. J Urol. 1998 May;159(5):1493-6.
10. Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BWG, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R. EAU Guidelines on Non-Muscle-invasive Urothelial Carcinoma of the Bladder: Update 2016. Eur Urol. 2017 Mar;71(3):447-461.
11. Nicholson RI, Gee JMW, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001;37:S9-15.
12. Colquhoun AJ, Mellon JK. Epidermal growth factor receptor and bladder cancer. Postgrad Med J. 2002 Oct1;78(924):584-9.
13. Weintraub MD, Vourganti S, Li Q, Apolo AB, Metwalli AR, Agarwal PK. Targeting the Epidermal Growth Factor Receptor in Bladder Cancer. J Carcinog Mutagen. 2013;4(2):1-7.
14. Wells A. The epidermal growth factor receptor (EGFR)-a new target in cancer therapy. Signal 2000;1:4-11.
15. Epstein JI, Amin MB, Reuter VR, Mostofi FK, and the Bladder Consensus Conference Committee. The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Am J Surg Pathol. 1998;22:1435-48.
16. Li W, Wang Y, Tan S, Rao Q, Zhu T, Huang G, Li Z, Liu G. Overexpression of Epidermal Growth Factor Receptor (EGFR) and HER-2 in Bladder Carcinoma and Its Association with Patients' Clinical Features. Med Sci monit. 2018;24:7178-85.
17. Rotterud R, Nesland JM, Berner A, Fossa SD. Expression of the epidermal growth factor receptor family in normal and malignant urothelium. BJU Int. 2005 Jun;95(9):1344-50.
18. Barua SK, Das N, Baruah SJ, Rajeev TP, Bagchi PK, Sarma D, Phukan M. Correlation of epidermal growth factor receptors core with prognostic variables in transitional cell carcinoma of urinary bladder among north-eastern Indian population. Int Surg J. 2018 Aug 25;5(9):3083-8.
19. Chow NH, Liu HS, Lee EI, Chang CJ, Chan SH, Cheng HL, et al. Significance of urinary epidermal growth factor and its receptor expression in human bladder cancer. Anticancer Res. 1997;17(2B):1293-6.
20. Badawy AA, El-Hindawi A, Hammam O, Moussa M, Helal NS, Kamel A. Expression of epidermal growth factor receptor and transforming growth factor alpha in cancer bladder: Schistosomal and non-Schistosomal. Curr urol. 2015;9(4):192-201.
21. Parvin M, Sabet-Rasekh P, Hajian P, Torbati PM, Sabet-Rasekh P, Mirzaei H. Evaluating the prevalence of the epidermal growth factor receptor in transitional cell carcinoma of bladder and its relationship with other prognostic factors. Iran J Cancer Prev. 2016 Feb;9(1):e4022.
22. Sriplakich S, Jahnson S, Karlsson MG. Epidermal growth factor receptor expression: predictive value for the outcome after cystectomy for bladder cancer?. BJU Int. 1999 Mar 1;83:498-503.
23. Neal DE, Sharples L, Smith K, Fennelly J, Hall RR, Harris AL. The epidermal growth factor receptor and the prognosis of bladder cancer. Cancer. 1990 Apr 1;65(7):1619-25.
24. Nguyen PL, Swanson PE, Jaszcz W, Aeppli DM, Zhang G, Singleton TP, et al. Expression of epidermal growth factor receptor in invasive transitional cell carcinoma of the urinary bladder: a multivariate survival analysis. Am J Clinical Pathol. 1994 Feb 1;101(2):166-76.