Original Article

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A 5 Year Analysis of Different Types of Urothelial Malignancies with Special Reference to The Expression Of EGFR in These Tumours

Shilpa K Narayanan[1]*, Praseeda I[2], S Vasudevan[1]

[1]Government Medical College, Thiruvananthapuram
[2]Travancore Medical College, Kollam

ABSTRACT

Background: Urothelial neoplasms are a heterogenous group of neoplasms with high propensity for recurrence. EGFR is a novel and potential biological marker for predicting the prognosis of urothelial malignancies. This study highlights the diagnostic and prognostic usefulness of EGFR as a phenotypic marker in urothelial malignancies. Thus, it warrants for anti-tumour therapy and prevention of recurrence of urothelial cancers by anti-EGFR therapy.

Methods: Current study includes 500 cases of urothelial tumours received in a major tertiary center in South Kerala. Based on the histopathological features these tumours were classified according to the WHO-2016 criteria. Proportion in each class of urothelial tumours during the study period was calculated. In addition to that, we also studied relation between EGFR expression and tumour grade and recurrence. Inclusion criteria: All consecutive cystoscopy biopsies, transurethral resection of bladder tumour (TURBT) and radical cystectomy specimens histologically diagnosed as urothelial malignancies of bladder. Exclusion criteria: All benign urothelial cases.

Result: Out of the 500 cases of urothelial malignancies included in the study, the non-invasive papillary urothelial carcinoma, low grade constituted the major part, the second being invasive urothelial carcinoma, high grade, followed by noninvasive urothelial carcinoma, high grade. A strong expression of EGFR was found to be significantly associated with higher tumour grade (P = 0.001) and recurrence (P = 0.010).

Conclusion: Histopathology supported by immunohistochemistry is a helpful tool in subtyping the various urothelial malignancies. EGFR can be considered as an adverse prognostic factor, the expression of which increases with tumour grade and stage. EGFR positivity may favor the use of targeted therapy in urothelial malignancies.

Keywords: *Urothelial Malignancies, Muscle Invasion, Prognosis, Staging, EGFR*

Introduction

Urothelial neoplasms are a heterogenous group of tumors with a high recurrent potential. It is the 9th most common cancer worldwide and accounts for about 2.25% cases annually [1]. The important predictors of prognosis of urothelial malignancies are grade and stage. Muscle invasion is considered as the single most important factor for determining disease prognosis in bladder cancer. If muscle invasion is present, then the therapeutic approach becomes more radical. The detection of abnormal expression of biological markers is under intense scrutiny in bladder cancer. It can serve as prognostic or predictive factors in bladder cancers. It is seen to be overexpressed in up to 74% of bladder cancer tissue specimens but has a low expression in normal urothelium.

EGFR is a novel and potential biological marker for predicting the prognosis of urothelial malignancies. Additionally, EGFR is regarded as an independent predictor of decreased survival and stage progression in bladder cancer [2]. A multitude of retrospective studies have shown a positive association between EGFR overexpression and adverse outcomes in superficial and muscle invasive diseases. It is considered as an adverse prognostic marker, the expression of which increases with tumour grade and stage. Thus, it warrants for anti-tumour therapy and prevention of recurrence of urothelial cancers by anti-EGFR therapy. The aim of my study is to histologically study urothelial malignancies and the expression of EGFR in them and to analyze their tumour grade and recurrence. The newer diagnostic modalities for early detection of cancer combined with EGFR inhibition therapy can impede the onslaught of urothelial cancers.

Materials and Methods

This is a descriptive study done in all consecutive cystoscopy biopsies, transurethral resection of bladder tumour (TURBT) and radical cystectomy specimens histologically diagnosed as urothelial malignancies of bladder in a major tertiary hospital in South Kerala, India, during the period from 2014-2018. A 5-year analysis of different types of urothelial malignancies based on their histopathology was done from 2014-2018 and to study the expression of EGFR in urothelial malignancies from 2016

to 2018 (2 years). It was given clearance by the Institutional Ethics Committee.

Clinical details of all cases of urothelial malignancies of bladder were collected using a structured proforma. Cases of urothelial malignancies of the urinary bladder from previous years were studied from their sections and histopathological reports. The paraffin embedded blocks were cut into 4-5µm thickness, and stained with hematoxylin and eosin. Based on the histopathological features these tumours were classified according to the WHO-2016 criteria.

Proportion in each class of urothelial tumours during the study period was calculated. In addition to that, the urothelial malignancy specimens received in the two-year study period was studied for EGFR expression by immunohistochemical analysis. The relation of EGFR with tumour grade and recurrence was analyzed. The staining condition was adjusted using placenta samples known to express EGFR as positive controls, Negative controls included omitting the primary antibody. The strength of EGFR immunostaining was graded as proposed by Atif Ali Hashmi et al[5].

Negative: No staining, unspecific staining of tumour cells or less than 10% staining. **Weak**: Membranous weak and incomplete staining of more than 10% of tumour cells. Strong: Strong and complete membranous staining of more than 10% of tumour cells.

All results and data were entered in Microsoft Excel and analyzed using the statistical software SPSS version.16. Ethical clearance was obtained from institutional ethics committee. Informed written consent has been obtained from the patients.

Result

Among the 500 cases studied, the age of patients ranged from 19 years to 89 years with a mean age of 62.5years. Of these, 421 (84.2%) were males and 79 (15.8%) were females. Thus, the male to female ratio was 5.3:1, showing a definite male preponderance.

At least one or combinations of the classic symptoms of urothelial malignancy i.e., hematuria, flank pain or irritable voiding symptoms were present in all the patients. Hematuria was the most common presenting symptom which was present in 453 (90.6%) patients.

Out of the 500 cases studied, 392 (78.4%) were solitary lesions, while 108 (21.6%) were multifocal. It was also observed that most of the multifocal lesions developed into invasive carcinomas. 65.8% were large tumours (>3cm) and 34.2% were small tumours (<3cm). Noninvasive urothelial carcinoma, low grade (39.8%) was found to be the most common lesion occurring in the bladder, followed by invasive urothelial carcinoma, high grade (32.2%). Squamous differentiation was seen in 28 cases (5.6%) , followed by glandular differentiation with 7 cases (1.4%) and sarcomatoid differentiation with 4 cases(0.8%).

Invasion of tumour into lamina propria was noted in 235 cases (47%). Out of the 500 cases, 41 samples didn't have muscle tissue and hence invasion could not be assessed; of which 254 cases (50.8%) were non muscle invasive and 205 cases (41%) were muscle invasive. 10 cases showed peri vesical fat invasion of tumour cells and 3 cases showed invasion to adjacent pelvic organs and lymph nodes. Among the 500 cases studied, 370 (74%) were primary cases while 130(26%) were recurrences. Assessing the clinical stage, 252 cases (56%) belonged to stage 0a followed by 191 cases (33.2%) which were of stage II category, i.e., they are tumours with muscle invasion.

Out of the 200 cases taken up for immunostaining procedure, it was observed that the invasive urothelial malignancies showed EGFR overexpression. It was observed that out of the 107 cases with lamina propria invasion, 56 cases (52.3%) showed strong EGFR expression, 18 cases showed weak positivity and 33 cases (30.8%) showed negative staining. 88 cases out of 200 showed muscularis propria invasion. Of which 56 cases (63.6%) showed strong positivity for EGFR. With a p value of 0.001, chi square testing showed significant association exists between EGFR positivity and lamina propria and muscle invasion. Among the 88 recurrent cases, 45 cases (55.5%) were positive and 36 cases (44.4%) were negative for EGFR immunostaining respectively.

Discussion

The present study attempted to assess the proportion of different types of urothelial malignancies and to study the expression of EGFR in these tumours. A total number of 500 cases of urothelial carcinomas were studied during a period of 5 years (2011 to 2018).

In this study, age at presentation ranged from 19-89 years. The mean age was 62.5 years. 63.8% of the patients belonged to the age group of 51-70 years. It was also observed that the incidence of urothelial carcinoma was uncommon below 30 years of age (1.8%). The findings are similar to a study by Karbakhsh. M et al[4] where the mean age was 62.4 years. Another study done on 960 cases of urothelial carcinoma by Atif Ali Hashmi et al[5], the peak incidence was seen at the age of 62.1 years**.** Male to female ratio was observed to be 5.3 : 1. This shows that there is a definite male preponderance. This observation is comparable to a study conducted by Shariat SF et al[3]

Table-1 Percentage distribution of different types of urothelial carcinomas

Histopathologic type	Number of cases	Percentage
PUNLMP	9	1.8
NIPUC, LG	199	39.8
NIPUC, HG	87	17.4
IUC, LG	44	8.8
IUC, HG	161	32.2

Table-2 Percentage distribution of cases according to EGFR expression

EGFR expression	Number of cases	Percentage
Strong	56	28
Weak	20	10
Negative	124	62
Total	**200**	**100**

Table-3 Association of EGFR expression with histopathological grade

Tumour grade	EGFR expression						X^2	p
	Strong		Weak		Negative			
	Counts	%	Counts	%	Counts	%		
PUNLMP	0	0	1	14.3	6	85.7		
NIUC, LG	0	0	0	0	75	100		
NIUC, HG	0	0	1	3.3	29	96.7	1.852	0.001
IUC, LG	4	19	8	11.9	7	33.3		
IUC, HG	52	77.6	10	47.6	7	10.4		

Table-4 Association of EGFR expression with invasion into muscularis propria.

		Muscle invasion			X^2	P value
		Present	Absent	Inconclusive		
EGFR expression	Strong	63.6%	0	2.2	1.423	0.001
	Weak	20.5%	2%	0		
	Negative	15.9%	98%	97.8		

Table-5 Association of EGFR expression with recurrence

		Primary	Recurrence	X^2	p value
EGFR expression	Strong	20.2%	39.5%	18.186	0.001
	Weak	5.9%	16%		
	Negative	73.9%	44.4%		

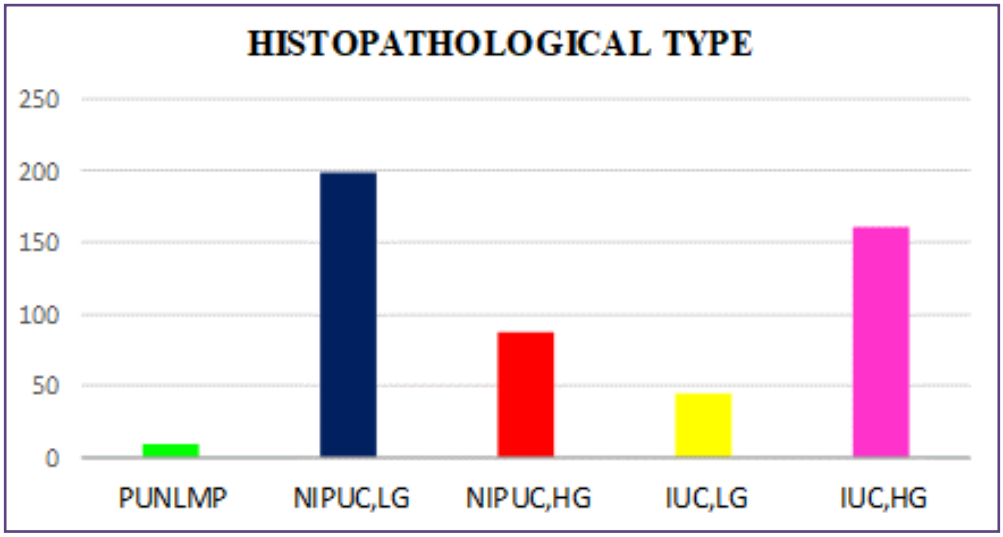

Fig.1: Frequency of different types of urothelial carcinomas.

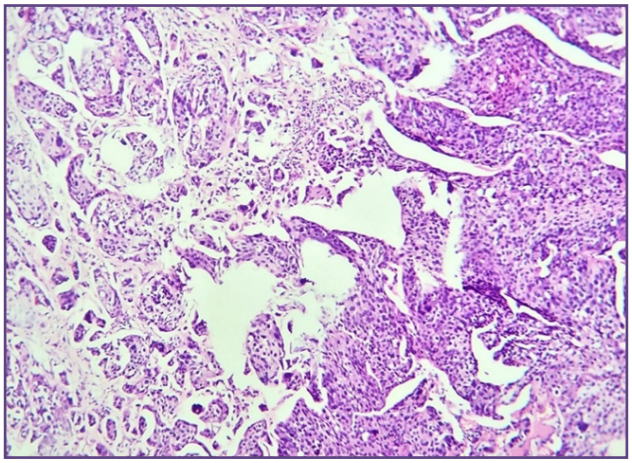

Fig. 2: H&E (40X) Invasive urothelial carcinoma, high grade with muscle invasion.

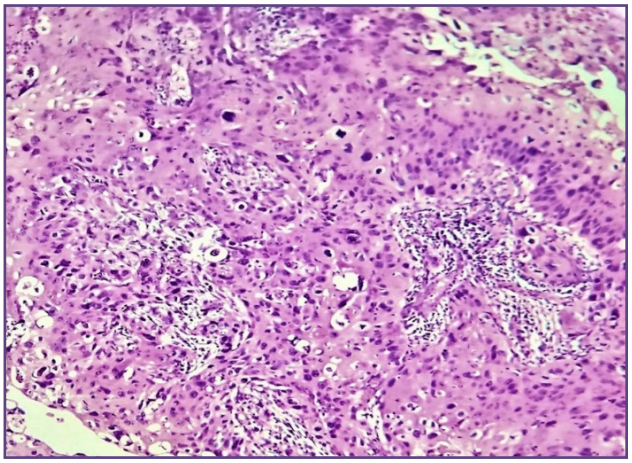

Fig. 3: H&E (40X) Invasive urothelial carcinoma, high grade with squamous differentiation.

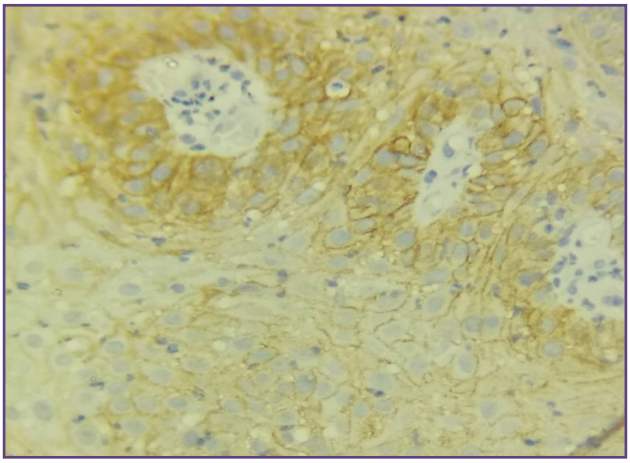

Fig. 4: EGFR (40X) Cytoplasmic and membranous positivity.

where the male to female ratio was 4:1. Another two studies by Rajesh Singh Laishram et al[6] and Lughezzani G et al[7] showed the sex ratio to be 3:1 and 1.5:1 respectively.

The current study showed hematuria is the leading clinical complaint in 90.6% of cases. This is comparable with a previous study by Siesen T et al[8] which had 85.6% cases. Irritative voiding symptoms were present in about 52.8% patients. Flank pain as a significant symptom was seen in 21.4% cases only.

The assessment of the number of tumours showed that 78.4% cases were unifocal and 21.6% were multifocal. This was true with the observations made by Munoz JJ et al[10] and Johansson SL et al[9] which showed a similar result of 77.5% and 74.1.5% unifocal tumours and 22.5% and 25.9% multifocal tumours respectively.

Out of the 500 cases 65.8% were large tumours (>3cm) and 34.2% were small tumours (<3cm). In the study by Herr HW et al[12] the result was 61.1% large tumours and 38.9% small tumours. The result was comparable with our study.

Among the 500 cases studied, it was noted that 57.2% cases were of non-muscle invasive type urothelial tumours. 32.2% were invasive urothelial carcinoma, high grade, 8.8% were invasive urothelial carcinoma, low grade and 1.8% cases were PUNLMP. The results are comparable with studies conducted by Mahesh Kumar et al[13] and Chang et al[11]. In this study we had also analyzed the incidence of histological differentiations that can occur in urothelial carcinoma. It was found that 5.6% cases showed squamous differentiation, 1.8% showed glandular differentiation and 0.8% cases had sarcomatoid differentiation. The histological differentiation has no prognostic significance.

In the Indian subcontinent, there is a higher incidence of high-grade tumours, and also most of the tumours are muscle invasive at the time of diagnosis. It can be attributed to late presentation of patients and environmental factors. Out of the 500 cases analyzed for invasion of tumour, it was found out that lamina propria invasion was noted in 235 cases (47%) and muscle invasion was seen in 205 cases (41%). It was also noted that 10 cases showed peri vesical fat invasion, and 3 cases showed invasion into prostate and regional lymph node. The results of our study is comparable with studies done by Dayalu S.L. Naik et al[14] and Atif Ali Hashmi et al. These factors are important in predicting the prognosis and may also be used for assessing the 5-year survival rate.

In the present study, 26% cases were recurrent tumours. Out of the 130 recurrent cases all of which occurred during the study period, 54.6% (71 cases) were invasive urothelial carcinoma, high grade, where as 9.2% (12 cases) were invasive urothelial carcinoma, low grade. The noninvasive carcinomas constituted 12.3% of the recurrent tumours. Atif Ali Hashmi et al, in his study had a recurrence rate of 43.1%, which was more than of our study. Since urothelial carcinoma shows high recurrence rate, as well as progression of noninvasive to invasive carcinoma at a much earlier stage compared to that of other malignancies, routine follow up is recommended. Among the recurrent tumours, 55% were invasive urothelial carcinomas, high grade and 32.3% were invasive urothelial carcinomas, low grade.

There is no association between age at presentation and gender with EGFR expression in our study. In the studies done by Atif Ali Hashmi et al and Dayalu S. L. Naik et al also noted that there existed no significant association between age at presentation and gender with EGFR expression.

Out of the 500 cases 252 (56%) belonged to stage 0a, followed by 191 (33.2%) cases were of stage II category. That is most of the tumours are those with invasion to muscle. Grading and staging of the tumours are the most important predictors of prognosis. On comparing results of our study with the study by Dayalu S.L. Naik et al, about 37.7 % belong to Stage II and 53% show Stage 0a. The results of both these studies are comparable.

Out of the 200 cases subjected to EGFR immunostaining, 56 cases (28%) showed strong positivity, 20 cases (10%) showed weak positivity and 124 cases (62%) showed negative results. The results are comparable with studies conducted by Atif Ali Hashmi et al and Nichols R I et[16]. Majority of cases which showed positive expression for EGFR were invasive tumours. Among this 77.6% were high grade tumours and 19% were low grade tumours. With a p-value of 0.001, it showed that there is a significant association between tumour grade and EGFR expression. The studies by Atif Alih Hashmi et al, Dayalu S. L Naik and Neal DE[1509] also showed significant association between tumour grade and EGFR expression. As in the literature the importance of EGFR expression is that, EGFR positive low grade tumours have a propensity for developing into high grade in a short duration. Hence the follow up of such patients will help the clinician to curtail the tumour progression at a very early stage.

Invasion of the tumours into lamina propria and muscularis propria was studied and it was found out that out of the 200 cases, 107 cases showed lamina propria invasion, of which 56 cases (52.3%) showed strong EGFR expression, 18 cases (16.8%) showed weak positivity for EGFR and 33 cases (30.8%) showed no staining for EGFR. With a p-value of 0.001, chi square testing showed a significant

association between EGFR expression and invasion of the tumour into lamina propria. It was also noted that majority of the tumours which showed EGFR positivity were muscle invasive ones. Out of the 88 muscle invasive tumours, 56 cases (77.6%) cases were strongly positive for EGFR. By doing Chi square testing, the p-value was deduced to be 0.001. There exists a significant association between invasion of the tumour into lamina propria and muscularis propria with EGFR expression**.** Once muscle invasion occurs, then radical cystectomy becomes the best modality of management. Here comes the possibilities of anti-EGFR targeted therapies which has a wide horizon in the years to come. The chance of tumour recurrence is also high once muscle invasion has occurred.

Out of the 200 cases subjected to EGFR staining, 88 of them were recurrent ones. 45 cases (55.5%) were positive and 36 cases (44.4%) were negative for EGFR immunostaining respectively. With a p-value of 0.010 the study showed significant association between EGFR expression and recurrence. In the study done by Atif Ali Hashmi et al also observed that a significant association exist between EGFR expression and recurrence.

Conclusion

Recently, EGFR has been identified as a biomarker of cancer stem cells in many malignancies, including urothelial carcinoma. From our study we conclude that the non-invasive papillary urothelial carcinomas constituted the major part of urothelial malignancies, the second being invasive urothelial carcinoma, high grade followed by invasive urothelial carcinoma, low grade. We also noticed a positive correlation between tumour grade and EGFR positivity. Strong positivity was seen in most of the high-grade tumours and weak positivity was seen in non-invasive low-grade tumours. An increase in EGFR staining was also noted with an increase in tumour stage. It was also noted that recurrent tumours showed strong EGFR immunostaining. However, no association was found between the age and gender with EGFR expression.

Thus, we conclude that EGFR can be considered as an adverse prognostic marker, the expression of which increases with tumour grade and stage. Such a positive relation may contribute to anti-tumour therapy and prevention of recurrence of urothelial cancers by anti-EGFR therapy. The newer diagnostic modalities for early detection of cancer combined with EGFR inhibition therapy can impede the onslaught of urothelial cancers.

Abbreviations

PUNLMP: Papillary Urothelial Neoplasm of Low Malignant Potential

NIPUC, LG: Non-Invasive Papillary Urothelial Carcinoma, Low Grade

NIPUC, HG: Non-Invasive Papillary Urothelial Carcinoma, High Grade

IUC, LG: Invasive Urothelial Carcinoma, Low Grade

IUC, HG: Invasive Urothelial Carcinoma, High Grade

EGFR: Epidermal Growth Factor Receptor

H & E: Hematoxylin & Eosin

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Competing Interests

Non declared

Reference

1. Mark Hurwitz, Philippe E. Spiess, Jorge A. Garcia. Urothelial and Kidney Cancers. Oncology 2016;10(12):56-6
2. Mellon K, Wright C, Kelly P, Horne CH, Neal DE. Long-term outcome related to epidermal growth factor receptor status in bladder cancer. Journal of Urology (1995); 153):919-25.
3. Shariat, S.F., et al. Gender differences in radical nephroureterectomy for upper tract urothelial carcinoma. World Journal of Urology 2011; 29(2): 481-4.
4. Karbakhsh M, Dabbagh N, Tabibi A, Shabani A, Akhavizadegan H. Age at diagnosis in bladder cancer: Does opium addiction play a role. Asian Pacific Journal of Cancer Prevention 2013; 14 (8): 4723-5.
5. Atif Ali Hashmi. Prognostic significance of epidermal growth factor receptor (EGFR) over expression in urothelial carcinoma of urinary bladder. BMC Urology2018; 18(1): 59-65
6. Laishram RS, Kipgen P, Laishram S, Khuraijam S, Sharma DC. Urothelial tumours of the urinary bladder in Manipur: A histopathological perspective. Asian Pacific Journal of Cancer Prevention 2012; 13(6): 2477-9.
7. Lughezzani, G., et al. Gender-related differences in patients with stage I to III upper tract urothelial carcinoma: results from the Surveillance, Epidemiology, and End Results database. Urology, 2010; 75(1): 321-6.

8. Seisen, T., et al. A Systematic Review and Meta-analysis of Clinicopathologic Factors Linked to Intravesical Recurrence After Radical Nephroureterectomy to Treat Upper Tract UrothelialCarcinoma. EurUrol, 201; 67(1): 1122-5.
9. Holmang S, Andius P, Hedelin H, Wester K, Busch C, Johansson SL. Stage progression in Ta papillary urothelial tumours: relationship to grade, immunohistochemical expression of tumour markers, mitotic frequency and DNA ploidy. American Journal of Pathology 2001; 165(4): 1124-30.
10. Munoz, J.J., et al. Upper tract urothelial neoplasms: incidence and survival during the last 2decades. Journal of Urology 2000; 164(2): 1523-5.
11. Chang SS, Hassan JM, Cookson MS, Wells N, Smith Jr JA. Delaying radical cystectomy for muscle invasive bladder cancer results in worse pathological stage. Journal of Urology 2003;170(41):1085–7.
12. Herr HW. A proposed simplified staging system of invasive bladder tumours. Urology International 1993; 50(15):17–20.
13. Kumar M, Yelikar B. Spectrum of lesions in cystoscopic bladder biopsies: A histopathological study. Journal of clinical and diagnostic research 2013; 7(8): 1609-11.16.
14. Dayalu S.L. Naik, Shashi Sharma, Amitabha Ray, Suresh Hedau. Epidermal Growth Factor Receptor Expression in Bladder Cancer. Indian Journal of Pathology2011; 27(2):208-214.
15. Neal DE, Sharples L, Smith K, et al. The epidermal growth factor receptor and the prognosis of bladder cancer. Cancer. 1990; 65(4):1619–1625.
16. Nicholson RI, Gee JMW, Harper ME EGFR. Cancer prognosis. European Journal of Cancer 2001;37(1):9–15.

***Corresponding author:**
Dr. Shilpa K Narayanan, Vishnu Bhavan, Ambancode, Peyyad (PO), Thiruvananthapuram-695573, Kerala, India
Phone: +91 9809539824
Email: drskn44@Gmail.Com

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