ORIGINAL ARTICLE

EVALUATION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) EXPRESSION IN UROTHELIAL CARCINOMA: A POSSIBLE PROGNOSTIC ROLE

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Background: Urothelial carcinoma is a subtype of urinary bladder cancer which carries a higher morbidity and mortality worldwide. It is estimated that 90% of the patients presenting to the hospitals with history of bladder cancer are diagnosed with urothelial carcinomas. Objective of the study was to evaluate the strength of EGFR expression and its prognostic role in urothelial carcinoma. It was a cross-sectional study conducted at the Rehman Medical Institute Peshawar from July 2022 to February 2023. **Methods:** A total of 98 specimens of patients diagnosed with urothelial carcinoma were examined in histopathology department. Patients with other malignancies were excluded. Demographic characteristics of participants, tumour grading, invasion of the surrounding structures and EGFR expression was recorded, and data analysis was performed with SPSS-23. **Results:** Mean age of the participants was 58.68±8.61 years. Gender distribution revealed 67(68.4%) males and 31(31.6%) females with a male to female ratio of 2.1:1. A total of 61 (62.2%) patients exhibited low grade of urothelial carcinoma while 37(37.8%) patients showed high grade malignancy. Expression of EGFR was not observed in 04 (4.1%) specimens while 62(63.3%) specimens revealed weak to moderate expression and 32(32.7%) showed strong expression of EGFR. Out of 61 patients with low grade of tumour, 04 (6.6%) patients exhibited no expression, 50(80%) patients exhibited weak to moderate expression and 07(11.5%) patients revealed strong expression of EGFR. Out of 37 patients with high tumour grade, 12(32.4%) revealed weak to moderate and 25 (67.6%) revealed strong expression of EGFR. Higher tumour grades and tumours with deeper penetration revealed a strong expression of EGFR with a *p*-value of <0.001. **Conclusion:** Strong expression of EGFR in tumours with higher grade validates its prognostic role and can be utilized for targeted therapy with potentially lifesaving consequences in patients presenting with urothelial carcinoma.

Keywords: Urothelial Carcinoma; EGFR; Bladder Cancer

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INTRODUCTION

Urothelial carcinoma is a subtype of urinary bladder cancer which carries a higher morbidity and mortality worldwide. It is estimated that 90% of the patients presenting to the hospitals with history of bladder cancer are diagnosed with urothelial carcinomas.[1] Several risk factors have been identified as the predisposing factors leading to urothelial carcinoma which includes smoking, genetic susceptibility, and gender predisposition. Males are three times at higher risk of developing urothelial carcinoma due to greater exposure to carcinogens as compared to females.[2] The high prevalence of the disease has led to an excessive burden of such patients on the health care system posing a great challenge to the multidisciplinary health care providers.[3] Urothelium is the upper epithelium lining the ureter and the bladder and a capability to renew after a period of 06 months. This makes the urothelium very prone to mutations leading to malignant transformation. Urothelial cancer is caused by multiple factors like smoking, indwelling catheters placed for a longer period, exposure to cyclophosphamide, schistosomiasis and occupational hazards like working in dye or rubber factories. The end result of all these risk factors is hyperplasia of the epithelial cells lining the urothelium and chronic inflammatory reaction which gives way to cancerous cells culminating in urothelial carcinoma.[4]

Prognosis of cancer greatly depends on the grading and staging of the tumor.[5] Depending on the presence or absence of papillae urothelial carcinomas can be either invasive(non-papillary) or noninvasive (papillary). Noninvasive neoplasms have greater chances of recurrence and can progress to invasion of the surrounding structures as compared to invasive

neoplasms however invasive neoplasms carries a greater risk of mortality.[4] The most important factor in the prognosis of the carcinoma is the muscle invasion by the tumour cells.[6] Molecular changes leading to urothelial carcinoma involves alteration in KMT2D and KTM6A genes which can modify the urothelial cells and enhance their mitotic activity. Additional alterations in genes like RB1, TP53, PIK3CA, or FGFR3 can further increase the mitotic activity of the cells resulting in urothelial carcinoma.[7]

Patients with urothelial carcinoma usually presents with hematuria, painful micturition or altered frequency of micturition. Based on history, examination and radiological findings on X-ray, computed tomography scan and Magnetic Resonance Imaging scans the diagnosis, grading and staging of urothelial carcinoma is made. A suspicion of urothelial cancer is usually followed by diagnostic cystoscopy and procuring a sample for biopsy examination. Epidermal growth factors are molecules which can promote growth of the epithelial cells. In a carcinoma the expression of epidermal growth factor receptors (EGFR) is highly increased, and this useful piece of information has led to the evaluation of EGFR in several carcinomas to assess the invasiveness and hence prognosis of cancer.[8,9] The rationale of this study is to assess EGFR expressions in patients diagnosed with urothelial carcinoma and its clinicopathological aspects which would help establish EGFR as prognostic marker and target for future therapy regimens.

MATERIAL AND METHODS

This cross-sectional study was conducted at Rehman Medical Institute Peshawar from July 2022 to February 2023 after obtaining approval of the Ethical Review Committee for a period.

With a 3% prevalence of bladder carcinoma [10] and employing a 5% level of significance with 95% power of test a sample size of 45 was calculated. Using a non-probability consecutive sampling technique total of 98 histopathology specimens of participants with urothelial lesions were reported as urothelial carcinoma by a consultant histopathologist.

Inclusion Criteria: Patients of either gender diagnosed with urothelial carcinoma were included in the study.

Exclusion criteria: Patients who were not willing, inadequate biopsy samples and those diagnosed with carcinomas other than urothelial carcinoma were excluded.

Total 98 biopsy specimens of candidates with suspected urothelial carcinoma were received in aseptic containers. All the specimens underwent tissue processing and paraffinization. Slides for eosin and haematoxylin staining were prepared followed by examination of sections by a consultant histopathologist. Histopathological assessment of the tumour grade, invasion of lamina propria, invasion of muscularis mucosa and lympho-vascular invasion was carried out. As per the records of each participant, demographic characteristics were recorded. Thereafter, immunohistochemical analysis was performed as per laboratory protocol using rabbit monoclonal primary antibody (Ref no 414R-28-ASR). Each slide was examined under microscope and EGFR was ascertained positive if the percentage of stained cells were equal to or greater than 10% showing a membranous and/or cytoplasmic pattern of staining. For slides with less than 10% staining of cells EGFR was recorded as negative. Intensity of EGFR staining was recorded as per a set criterion of weak, moderate or strong membranous/cytoplasmic staining of tumour cells. Overall immunoreactivity of the EGFR staining was recorded as 0 in the absence of staining or light staining in <10% of tumour cells (Figure-1) while 1+, 2+ and 3+ was recorded in the presence of weak, moderate or strong staining respectively in ≥10% of tumour cells (Figure-2). For ease of analysis all specimens recorded as 0 were represented as no expression, +1 and +2 specimens as weak to moderate expression and +3 was presented as strong expression of EGFR.[11]

analysis was performed using SPSS version 23. For qualitative variables frequency and percentages were computed while mean±S.D values were used for quantitative variables. Qualitative variable comparison was performed by using chi square test while quantitative variables were analyzed using independent t-test and Man Whitney U-test. A *p*-value of <0.05 was considered as significant.

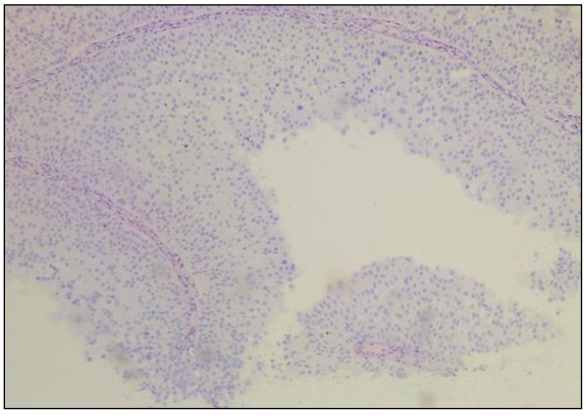

Figure-1: Absence of EGFR staining is seen in low grade Urothelial Carcinoma

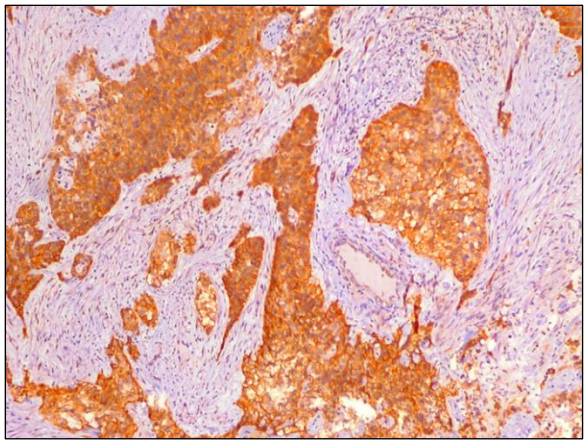

Figure-2: Strong staining of EGFR is seen in high grade Urothelial Carcinoma

RESULTS

A total of 98 participants were included in the trial with a mean age of 58.68 ± 8.61 years. Demographic details as presented in Table-I show a male to female ratio of 2.1:1. Of a total of 98 cases, 61(62.2%%) patients exhibited low grade of urothelial carcinoma while 37(37.8%) patients had high grade of urothelial carcinoma. Expression of EGFR was not observed in 04(4.1%) specimens while 62(63.3%) specimens revealed weak to moderate expression and 32 cases (32.7%) showed strong expression of EGFR. Demographic characteristics of the patients are shown in Table-1. Out of 61 patients with low grade of tumor, 04(6.6%) patients exhibited no expression, 50(80%) patients exhibited weak to moderate expression and 07(11.5%) patients revealed strong expression of EGFR. Out of 37 patients with high tumor grade, 12(32.4%) revealed weak to moderate and 25(67.6%) revealed strong expression of EGFR. Higher tumor grades and deeper penetration of the tumor revealed significant results with a *p*-value of <0.001. Correlation of EGFR expression with histopathological findings is shown in Table-2.

Table-1: Demographic characteristics of participants (n=98)

Variables		n (%)
Age	<55years	33 (33.7%)
	>55years	65 (66.3%)
Gender	Male	67 (68.4%)
	Female	31 (31.6%)
Tumour Grade	Low	61 (62.2%%)
	High	37(37.8%)
EGFR expression	No expression	04 (4.1%)
	Weak to Moderate expression	62 (63.3%)
	Strong expression	32 (32.7%)
Invasion	No invasion	19 (19.4%)
	Lamina propria invasion	54(55.1%)
	Lamina Propria and muscle invasion	10(10.2%)
	Lamina propria and lymphovascular invasion	08(8.2%)
	Lamina propria, lymphovascular and muscle invasion	07(7.1%)

Table-2: Correlation of EGFR expression with histopathological findings

Variables		No expression of EGFR n (%)	Moderate expression of EGFR n (%)	Strong expression of EGFR n (%)	*p*-value
Age	**<55years**	03(9.1%)	20(60.6%)	10(30.3%)	0.202
	>55years	01(1.5%)	42(64.6%)	22(33.8%)	
Gender	**Male**	04(6%)	39(58.2%)	24(35.8%)	0.187
	Female	00	23(74.2%)	08(25.8%)	
Tumour grade	**Low**	04(6.6%)	50(80%)	07(11.5%)	<0.001
	High	00	12(32.4%)	25(67.6%)	
Invasion	**No invasion**	04(21.1%)	15(78.9%)	00	<0.001
	Lamina propria invasion	00	37(68.5%)	17(31.5%)	
	Lamina Propria and muscle invasion	00	08(80%)	02(20%)	
	Lamina propria and lympho-vascular invasion	00	00	08(100%)	
	Lamina propria, lympho-vascular and muscle invasion	00	01(14.3%)	06(85.7%)	

DISCUSSION

The cross-sectional study was conducted to evaluate the strength of EGFR expression and to study the clinicopathological parameters in relation to EGFR expression. Low to moderate expression of EGFR was seen in 62(63.3%) while 32(32.7%) revealed strong

EGFR expression. Total high-grade carcinomas were 37 out of 98 specimens and 12(32.4%) showed low to moderate expression of EGFR as compared to 25(67.6%) specimens revealing strong expression of EGFR. In our study the EGFR expression was stronger for deeper and more invasive tumours as compared to tumours with no invasion of the underlying structures. In a similar study based on EGFR expression in urothelial carcinoma the expression of EGFR was stronger when tumours had a higher grade of morphology 25(75.8%) as compared to 08(24.2%) specimens with low grade histological characteristics. Similarly, tumours invading lamina propria and muscularis mucosa revealed strong EGFR expression as compared to non-invasive tumors.[12]

Advances in the field of histopathology have paved a pathway for a number of markers which can be used as prognostic indicators in patients presenting with urothelial carcinoma. In addition to EGFR, PKM2, CD117, VEGF and EMMPRIN also revealed their positive role as prognostic markers in urothelial cancer.[13] The expression of these markers play a vital role in guidance to early interventional therapy by the use of pharmacological agents which can block the receptors and decrease the rapid mitotic activity of the tumour cells. Another trial was conducted to evaluate the expression of Extracellular matrix metalloproteinase inducer (EMMPRIN) and EGFR in urothelial carcinoma. Invasion of the muscular layer by urothelial carcinoma is not a sign of good prognosis and in this analytical observational study a positive correlation was found between EMMPRIN and EGFR. Urothelial carcinomas with invasive nature colonizing the muscular layer revealed over expression of both EMMPRIN and EGFR.[14]

Several factors have been under trials for prognostic roles in urothelial carcinoma. Another trial revealed that when Vascular endothelial growth factor (VEGF), EGFR and prostate specific membrane antigen (PSMA) were evaluated for lymph node metastasis both PSMA and EGFR did not produce significant results. This concluded that only VEGF is a reliable marker signifying nodal metastasis in urothelial carcinoma.[15] The significant prognostic role of EGFR in another study revealed that tumours with higher tumour grade, increased recurrence, invasion of lamina propria or muscularis layer exhibit strong expression of EGFR.[16]

Similar to EGFR, pyruvate kinase M2 (PKM2) expression in urothelial carcinoma is of paramount significance. El-Sheikh P Et al investigated EGFR and PKM2 expression in urothelial carcinoma and its role as prognostic markers. Both of them showed strong expression in urothelial carcinoma with higher tumour grade or invasion of the lamina propria and muscle layer. A positive correlation between the two signified the equal importance of PKM2 as a prognostic marker in urothelial carcinoma.[17] Similar to the results produced by our study multiple trials have proven that expression of EGFR in urothelial carcinoma bears a reliable prognostic significance.[18,19]

Recent advances in novel therapies have paved a way for strategies which can inhibit the expression of EGFR in cancerous cells. These monotherapies or combination therapies include EGFR inhibitors, RNA interference, epigenetic modulation or a combination of such therapies which can suppress the intensity of EGFR expression. Resultantly such evolving therapies can suppress the ability of rapidly dividing cancer cells and pose a better outcome in patients diagnosed with cancer.[20,21]

The role of EGFR in other cancers have also been validated by several trials and use of EGFR as a prognostic marker in breast, colorectal, gall bladder, prostate and ovarian cancer cannot be negated. This study proves the effective prognostic role of EGFR in our locoregional patients presenting with urothelial carcinoma hence a guideway to early EGFR inhibitor therapy for suppression of cancerous cell division resulting in better prognosis and outcome.

CONCLUSION

Strong expression of EGFR in tumors with higher grade and deeper penetration validates its prognostic role and can be utilized for targeted therapy with potentially lifesaving consequences in patients presenting with urothelial carcinoma.

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Limitations of the Study: None

Conflict of Interest: None

AUTHORS' CONTRIBUTION

SB: Conceived, designed and did statistical analysis, editing and responsible for integrity of research. AS, FJ, ZB, MK, MTK: Helped with data collection, statistical analysis and write-up. IMK: Review and final approval of the manuscript.

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