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Immunohistochemistry Study Urothelial Carcinoma using Tumor Markers (EGFR, EMA and CD117)

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ABSTRACT

The aim of the present is to determine the immunohistochemical expression of EGFR, EMA and CD117 in case of urinary bladder transitional cell carcinoma and their relation with staging of tumor and its impact on further prognosis. EGFR immunohistochemical study showing (61.5 %) positive in case of urinary bladder carcinoma with significant difference between staging of tumor (p value 0.019) with signification expression between low grade and high grade tumor with P value 0.002 (6.2%) positivity in high grade versus (15.4 %) positivity in low grade). EMA showing strong positivity in urothelial carcinoma 96.2% without significant difference between staging and grading of tumor with p valve of (0.57) and (0.227) respectively CD117 immunohistochemical study showing total positive cases of urinary bladder carcinoma of 69.2% with significant difference in comparison with staging and grading of tumor with (P-value=0.00) for each respectively.

Our result demonstrate that EGFR and CD 117 immunohistochemical staining showing strong positivity in majority of urothelial carcinoma cases with significant relation to both pathological grading and staging of tumor. Application of both markers may play a role to give idea about behavior of tumor and possibility of further recurrence with and even direct therapy application EMA showing strong positive expression which give idea about tumor origin from epithelial lining. It had no benefit in assessment of tumor grading and staging

Keyword: urothelial carcinoma, EGFR. EMA, CD117

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INTRODUCTION

Urinary bladder cancer is the most common malignancy in urogenital tract and ranked as 9th in cancer frequency worldwide, it more common in males than in females [1]. Urothelial carcinoma represents about 90% of malignant bladder tumors [2]. Fortunately this tumor can be diagnosed at an early stages because of symptom that are associated with it and this give a good opportunity for successful treatment and decreased risk of recurrence but it mandate a good follow up protocol [3]. Macrohematuria and dysuria are most frequent symptom in this tumor and these tumors and usually non-muscle invasive but carry high risk of recurrence and this make an effective monitory necessary [4-5], standard methods to diagnose urothelial cancers including urine cytology, imaging tests and cystoscopy evaluation of urinary bladder [2-3]. Urine marker was increasingly used for diagnosis of urothelial cancers in attempts to replace cystoscopy as this procedure is costly and uncomfortable to the patients [6]. Molecular evaluation of urothelial cancer becomes increasingly mandatory to predict the biological behavior of this tumor. Increasing biological aggressiveness of this tumor reflected by higher grade and advance stage were associated with different genetic aberrations as microsatellite instability and loss of heteozygosity [7]. Endothelial growth factors receptor expression normally limited to the basal layer of the urothelial it over expressed in all cell layers in urothelial carcinoma [8-9]. This study was designed to investigate the immunohistochemical expression of tumor marker (EGFR, EMA, and CD117) in urothelial carcinoma and to predict its importance in predicting the possible biological behavior of this tumor.

MATERIAL AND METHOD

This is a cross-sectional study performed in department of pathology/ faculty of medicine/university of Kufa in a period extending from January, 2018 to January, 2020. The study was approved by ethics committee of university of Kufa. All patients involved in study were informed and they signed and informed consent. This study includes 78 formalin-fixed and paraffin embedded block from patients having urothelial carcinoma. These blocks were randomly collected from the university of Kufa teaching hospital and pathology laboratories in Al-Najaf province by persons who are blind to the aim of study. All cases were stained by hematoxylin and eosin stain and the diagnosis were confirmed by two independent pathologist. Grading and staging were estimated at the same time according to AJCC cancer staging [10]. All cancers were stained for immunohistochemical markers (EGFR, EMA, and CD117)

using immunohistochemistry protocol using envisions method by Dako kit manufactures instructions [11]. Immunohistochemical technique done by used formalin fixed and paraffin embedded blocks tissue by sliced 4 um thickness and putted on slides positive charged, the slides deparaffanized by heating in 60ᶜ for 70 min in an oven, then were dew axed with xylene twice for 5 min and rehydrated gradually in alcohol as series concentration((100%,90% and 70%) three min, after that the slides were washed in distilled water then immersed the slides with high pH retrieval by heating in water path at 95ᶜ for 20 min followed cool at room temperature. Following blocking of endogenous peroxidase activity for 5 min and immediately washed with phosphate-buffered saline. The primary antibody (EGFR, CD117 and EMA) diluted at working concentration and used on the slides for 20 min. The enzyme HRP was added for 20 min and also washed with buffer. Diaminobenzidine (DAB) was diluted with substrate buffer and used as chromogen for 10 min and the sections were subsequently counterstained with hematoxylin for 2 min, then dehydrated by used three up graded of alcohol concentration, cleared with xylol, and mounted by DPX. Immunohistochemical staining was examined under bright-field microscopy (Ordinary optical microscope OlymPusBX40, Japanese Olympus Company). Statistical analysis was done using statistical package of social sciences (SPSS), version 23. Qi-square test and exact test were utilized for data analysis and p-value less than 0.05 regarded significant.

RESULTS

This is a cross sectional study performed at the department of pathology and forensic medicine – faculty of medicine-university of Kufa. This study showed that the majority of cases display positive staining for EMA. All case of T1 are positive, in T2 tumor only 3 cases are negative while 39 cases show positive staining. All cases of Ta were positively stained for EMA and this is also true for T4 tumors, table (1) and figure (1).

Table 1: The distribution of EMA according to stage of the tumor, all the studies samples were positive of this marker in all stages of the tumor

			EMA			P value
			negative	positive	Total	
stage	T1	Count	0	6	6	
		% of Total	0.0%	7.7%	7.7%	0.57
	T2	Count	3	39	42	
		% of Total	3.8%	50.0%	53.8%	
	T4	Count	0	6	6	
		% of Total	0.0%	7.7%	7.7%	
	Ta	Count	0	24	24	
		% of Total	0.0%	30.8%	30.8%	
Total		Count	3	75	78	
		% of Total	3.8%	96.2%	100.0%	

This study showed that all low grade tumor stained positively for EMA while 3 out of 48 cases of high grade tumor display that marker. There were no significant differences in the patterns of staining of this marker in respect of tumor grade (p=0.227) (table-2)

Table 2: The distribution of EMA according to grade of the tumor, all the studies samples were positive of this marker in all stages of the tumor

			EMA			P value
			negative	Positive	Total	
grading	high grade	Count	3	45	48	
		% of Total	3.8%	57.7%	61.5%	0.227
	low grade	Count	0	30	30	
		% of Total	0.0%	38.5%	38.5%	
Total		Count	3	75	78	
		% of Total	3.8%	96.2%	100.0%	

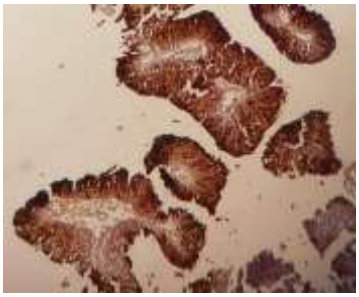

Figure 1: High grade papillary urothelial carcinoma with strong EMA luminal stain of superficial cells and cytoplasmic stain for intermediate and basal cells (*100)

This study showed that all cases of T1, and T4 were stained positively of EGFR while 24 out of 42 T2 and 12 out of 24 in Ta display positive results. There were significant difference in EGFR staining patterns regarding stage of the tumor (p=0.019), table (3) and figure (2).

Table 3: Show the distribution of EGFR according to stage of the tumor

			EGFR			P value
			negative	positive	Total	
stage	T1	Count	0	6	6	
		% of Total	0.0%	7.7%	7.7%	0.019
	T2	Count	18	24	42	
		% of Total	23.1%	30.8%	53.8%	
	T4	Count	0	6	6	
		% of Total	0.0%	7.7%	7.7%	
	Ta	Count	12	12	24	
		% of Total	15.4%	15.4%	30.8%	
Total		Count	30	48	78	
		% of Total	38.5%	61.5%	100.0%	

Our study showed that 36 cases out of 48 in high grade tumor display positive staining for EGFR while 12 out of 30 cases showed stained positively in low grade tumor with significant differences between the grades table-4

Table 4: Show the distribution of EGFR according to grade of the tumor

			EGFR			P value
			negative	positive	Total	
grading	high grade	Count	12	36	48	
		% of Total	15.4%	46.2%	61.5%	0.002
	low grade	Count	18	12	30	
		% of Total	23.1%	15.4%	38.5%	
Total		Count	30	48	78	
		% of Total	38.5%	61.5%	100.0%	

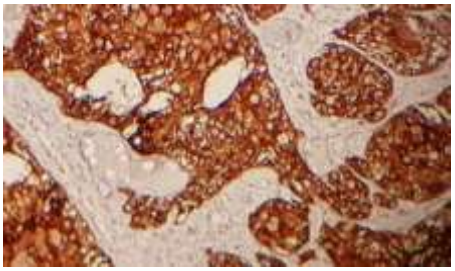

Figure 2: High grade papillary urothelial carcinoma with strong diffuse membranous staining EGFR (X100)

Current study showed that all cases of T1 and T4 stage are positively stained for CD117 while 36 out 42 in T2 cases and 6 out of 24 cases in Ta tumors showed positive staining for this marker with significant differences in the staining pattern regarding stage of the tumor, table (5) and figure (3).

Table 5: Show the distribution of CD117 according to stage of the tumor

			CD117			P value
			negative	positive	Total	
stage	T1	Count	0	6	6	0.000
		% of Total	0.0%	7.7%	7.7%	
	T2	Count	6	36	42	
		% of Total	7.7%	46.2%	53.8%	
	T4	Count	0	6	6	
		% of Total	0.0%	7.7%	7.7%	
	Ta	Count	18	6	24	
		% of Total	23.1%	7.7%	30.8%	
Total		Count	24	54	78	
		% of Total	30.8%	69.2%	100.0%	

This study showed that 42 cases out of 48 cases of high grade tumor and 12 out of 30 in low grade tumor are positively stained for CD117. There were significant differences in CD117 staining in respect to grade of the tumor.

Table 6: Show the distribution of CD117 according to grade of the tumor

			CD117			P value
			negative	Positive	Total	
grading	high gra	Count	6	42	48	0.000
		% of Total	7.7%	53.8%	61.5%	
	low grad	Count	18	12	30	
		% of Total	23.1%	15.4%	38.5%	
Total		Count	24	54	78	
		% of Total	30.8%	69.2%	100.0%	

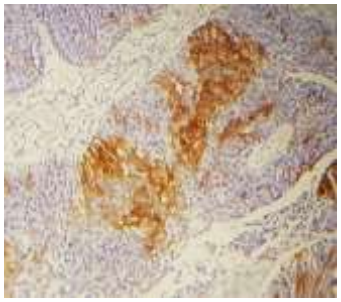

Figure 3: Low grade papillary urothelial carcinoma with moderate membranous and cytoplasmic staining for CD117 (magnification (X100)

DISCUSSION

Cancer of urinary bladder is considered as the 5th most common malignant tumor in the western population. The mortality rate is high in advanced stages of the tumor. However, a slight improvement in the survival was noticed in the last three decades [12]. The family of EGFR in human composed of 4 members EGFR, HER3, HER2 and ERBB2. This in-depth study were performed to investigate the expression of EGFR in bladder carcinoma and to correlate this marker expression with prognostic parameters. These are transmembrane proteins. Structurally they had 3 important parts: a hydrophobic transmembrane region, extracellular domain for ligand binding, and intracellular domain [13], binding of EGF to its binding site lead to activation of these tyrosine kinases. In the normal state two receptors dimerize after binding of EGFR to EGF, this will trigger autophosphorylation and activation of the dimer [14]. Active receptors in turn recruits proteins leading to phosphorylation and activation of RAs, this will witch on the MAPK/ERK complex and traducing a mitogenic signals for the downstream pathway [15]. This had in important contribution in cellular proliferation, differentiation, apoptosis and angiogenesis [16-18]. On the other hand EGFR is expressed on epithelial cells. It was involved in carcinogenesis through regulation of cellular mortality, cancer invasion, metastases and apoptosis [19]. Solid tumors in human shower expression of this receptor as demonstrated in cancers of bladder, breast, stomach and colorectal cancers [20]. In invasive and metastatic cancers, EGFR represent the corner stone in the signaling pathway that regulate such cancers properties [16]. Expression of such protein induces cellular migration which was prerequisite for metastasis of the tumor [21]. Amplification of EGFR in bladder cancer well associated with aggressive tumor behavior as it was found in high stage and/or high grade tumors [22-24]. The present study showed that 48 out

of 78 case show over expression of EGFR in different stages between the patients enrolled in this study with significant difference in respect to this important prognostic parameter (p=0.019). This indicated that this protein had a role in the biological progression and it may be a part of the story in of the biologic behavior of the tumor. This study also shows that there were significant differences in expression of EGFR between low grade and high grade tumor. This denotes that this protein had a role in the differentiation of the tumor and in turn affects the prognosis and possibly the tumor management.

Epithelial membrane antigen is surface glycoproteins. It was expressed on epithelial cells and on tumor of epithelial origin. It had and regulatory and protective functions. It represents a barrier on apical portion of epithelial cells surfaces. Bladder cancer cells express EMA both in deeper and superficial layers and this in contrast to normal urothelium that express EMA in upper layer only. High grade tumors characteristically showed irregular EMA expression [25]. This study clarify that 75 cases out of 78 cases show positive staining results for EMA. No difference in the marker expression pattern were observed in respect to grade (p=0.227) and stage of tumor (p=0.57). This pattern of staining denotes that EMA is an important marker in clarifying the epithelial differentiation but it may not play an important role in the biologic progression of the tumor. CD117 is tyrosine kinase receptor [26]. It act by binding to stem cells factors, it had a role in cellular adhesion, apoptosis, migration and differentiation [27]. In our study, 54 cases showed a positive immunohistochemical staining for CD117. There were significant differences this immunohistochemical marker expression in respect to stage and grade of tumor. This reflect the importance of this marker and precisely C-kit mutation in the development and progression of the tumor

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DISCLOSURE OF INTEREST

The authors report no conflict of interest

DATA AVAILABILITY STATEMENT

The data support the findings of this study are available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

ASJ and QMT: conception and work design.
ASJ, HSH and ICA: data analysis and draft writing.
ASJ, HSH and ICA: data analysis and interpretation.
QMT and AQ: Drafting the work or revising it critically for important intellectual content
All authors approved the final version to be published.

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