ORIGINAL ARTICLE

CD 10 Expression Intensity in Various Grades and Stages of Urothelial Carcinoma of Urinary Bladder

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ABSTRACT

Objective: To evaluate CD10 expression in urothelial carcinoma of the urinary bladder and the association of immunohistochemical (IHC) CD10 expression intensity with grade and stage.

Study Design: Descriptive cross-sectional analytical study.

Place and Duration of Study: Armed Forces Institute of Pathology, Rawalpindi, from January to December 2011.

Methodology: Fifty consecutive cases of urothelial bladder carcinomas, obtained through transurethral resections, were included in this study. Hematoxylin-eosin (HE) stained sections from each case were re-evaluated histopathologically according to WHO 2004 grading system. The TNM system was used for pathologic staging. On selected slides IHC CD10 marker was applied and a semiquantitative scoring for its expression based on the percentage of positive cells and intensity was performed. Data was entered and analysed on SPSS version 17. Fisher's exact test was used to compare grades, stages of urothelial carcinoma with CD 10 expression and age groups. $P < 0.05$ was taken as level of significance.

Results: Urothelial carcinoma was more common in males. The male to female ratio was 9:1. The older patients > 50 years had higher grade and stage as compared to the younger patients. All cases of high grade urothelial carcinoma showed higher positivity for CD 10. Twenty cases (86.95%) of high grade urothelial carcinoma were positive with +2 immunostaining while 3 cases (13.04 %) were positive with +1 staining. None of the tumors of stage pTa was positive for CD 10 expression. Of all patients with stage pT 1 tumor, 1 case (5.3%) was CD 10 negative and 17 cases (89.9%) were CD 10 positive having +1 staining with 5 - 50% staining and 1 case (5.3%) had +2 staining with more then 50% expression. Out of all patients with stage pT 2, no tumor was CD 10 negative, 3 (13.6%) patients were CD 10 positive with +1 staining and 19 (86.4%) with stage pT 2 tumor had stained positive with +2 staining.

Conclusion: CD 10 expression was greater in high grade and invasive urothelial carcinomas; it may be associated with tumor progression in bladder cancer pathogenesis.

Key Words: *Grade. Invasive urothelial carcinoma. Immunohistochemistry. CD 10 expression. Bladder carcinoma.*

INTRODUCTION

Cancer of the urinary bladder represents the ninth most common cause of cancer worldwide and the 13th most common cause of cancer deaths.[1] It is the fourth most common malignancy in men and tenth most common malignancy in women in United States. Bladder carcinoma is more common in whites, with male to female ratio of 3 : 1, and the median age at diagnosis is 68 years. According to AFIP data urothelial carcinoma of bladder is the seventh most common malignancy in both males and females and constitutes 93.4% of all bladder cancers. In approximately 75 - 85% bladder cancer patients, the disease is confined to the mucosa and has a prolonged clinical course with multiple recurrences after local resection without tumor progression.[2] In contrast, a smaller but significant percentage of patients have advanced and muscle-infiltrative tumor at the time of diagnosis.[3]

Different parameters determine the prognosis of bladder carcinoma, including stage, grade, patient's age, and lymph node status. Prolonged survival in most patients with superficial cancers is achieved by transurethral resection (TUR) with or without intravesical chemo-therapy. Nonetheless, these patients still have a high risk of recurrence following initial resection.[4] The major prognostic factors in carcinoma of the bladder are the depth of invasion into the bladder wall and the degree of differentiation of the tumor. However, there is no reliable parameter predicting the risk of recurrence or progression. Molecular markers are, therefore, required to estimate the individual prognosis of patients as well as for effective diagnosis and treatment.

The CD 10 is a single-chain, 90-110-kDa cell surface zinc dependent metalloprotease that inactivates various bioactive neuropeptides.[5] In addition to its enzymatic function, CD10 protein has a direct role in signal transduction pathways that regulate cell growth and apoptosis and because of its structural similarity to the matrix metalloproteases in the stroma, CD10 is also thought to affect invasion and metastatic potential of

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tumor cells by altering the cellular microenvironment.[6] This was initially discovered on the surface of acute lymphoblastic leukemia cells, and considered to be a tumor-specific antigen.[7] This marker has a neutral endopeptidase activitiy and is known to regulate biological activities of peptide substrates. There are recent evidence demonstrating a correlation between apoptosis and CD10 expression.[8,9]

There are no studies in Pakistan regarding CD 10 expression in urothelial carcinoma and very few studies internationally investigated its expression.[10] The rationale of this study is to evaluate CD10 (common acute lymphocytic leukemia antigen) immunohistochemical expression in urothelial carcinoma of the urinary bladder and to associate this expression with various histo-pathological parameters including grade and stage, thus contributing as a prognostic factor.

METHODOLOGY

Fifty consecutive cases of papillary urothelial neoplasms of the urinary bladder from transuretheral resection of bladder tumor (TURBT) were included in this study at Armed Forces Institute of Pathology (AFIP) from January to December 2011. Haematoxylin and eosin stained sections from formalin-fixed, paraffin-embedded material, were re-evaluated histopathologically. Tumors were subclassified as papillary neoplasm of low malignant potential, as low-grade papillary carcinoma, or as high-grade papillary carcinoma according to the WHO 2004 grading system.[11] The TNM system was used for pathologic staging: pTa, non-invasive papillary urothelial carcinoma; pT1, tumor invades sub-epithelial connective tissue; pT2, tumor invades muscularis propria, and pT3, tumor invades perivesical tissue.[12]

The inclusion criteria comprised of all cases of papillary neoplasm of low malignant potential, low grade and high-grade papillary urothelial carcinoma with or without invasion. All benign lesions including papilloma and poorly fixed specimen were excluded. Immuno-histochemistry was performed using a streptavidin-biotin-peroxidase technique with a monoclonal antibody to CD10. Five -µm sections from paraffin- embedded samples were cut on poly-L-lysine coated slides, deparaffinized in xylene, and then dehydrated. For antigen retrieval, the slides were treated by microwave heating in citrate buffer (pH 6.0) for 10 minutes. Three percent hydrogen peroxide was used for blocking endogenous peroxidase activity. The sections were incubated with primary antibody including CD10 at 1:50 dilution for one hour at room temperature. After washing in phosphate buffered saline, the samples were incubated with a biotin conjugated secondary antibody and then incubated using streptavidin-biotin system for 30 minutes at room temperature. The reactions became visible after immersion of the specimens in diaminobenzidine tetra hydrochloride. The sections were counterstained with Hematoxylin, then rinsed and mounted. Sections from non-neoplastic bladder mucosa were included as controls. Additional sections of renal tissue were also used as positive control. Staining of the cell membrane and/or cytoplasm was considered positive expression. A semi quantitative scoring based on the percentage of positive cells was performed according to the following staining criteria: - negative (< 5% of tumour cells were positive); 1+ (5 - 50% of tumour cells were positive); and 2+ (> 50% of tumour cells were positive).[5]

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 17. Qualitative variables like grades and stages of urothelial carcinoma along with CD 10 expression were calculated in terms of frequency and percentages. Fisher's exact test was used to compare grades, stages of urothelial carcinoma with CD 10 expression and age groups. $P < 0.05$ was taken as level of significance.

RESULTS

Fifty cases of urothelial carcinoma were included in this study. Majority of patients i.e. 40 (80%) were more than 50 years old while 10 (20%) were younger than 50 years. Predominant population was male 45 (90%) and only 5 (10%) females. Number of older patients suffering from high grade and stage urothelial carcinoma was higher. Amongst older patients, 21 (52.5%) had high grade papillary urothelial carcinoma, 18 (45%) had low grade papillary urothelial carcinoma while 1 (2.5%) had papillary neoplasm of low malignant potential. In young patients 4 (40%) had papillary neoplasm of low malignant potenial, 4 (40%) had low grade papillary urothelial carcinoma and 2 (20%) had high grade urothelial carcinoma. This relationship of age groups with tumor grades was also statistically significant ($p < 0.001$, Table I).

On histopathological examination, 9 (18%) had non-invasive tumors (pTa), further 19 (38%) had invasion restricted to lamina propria (pT1) and remaining 22 (44%) had advanced stage tumor invading lamina propria as well as muscles (pT2). More patients i.e. 23 (46%) had high grade papillary urothelial carcinoma, another 22 (44%) had low grade papillary urothelial carcinoma and only 5 (10%) had papillary neoplasm of low malignant potential (Table I).

When tumors of different grades were analyzed for expression of CD 10, it revealed that none of the papillary neoplasm of low malignant potential was positive for CD 10 expression. Of all patients with low-grade papillary urothelial carcinoma, 5 (22.7%) were CD 10 negative and 17 (77.3%) were CD 10 positive with level one expression i.e. 5 - 50% staining. All patients with high grade urothelial carcinoma had stained positive with CD 10. Most of the patients with high grade

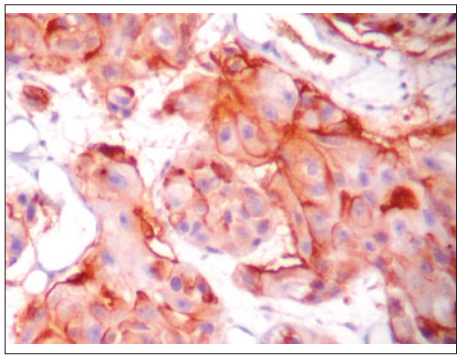

Figure 1: Diffuse CD10 immunostaining (+2) in high grade urothelial carcinoma x400.

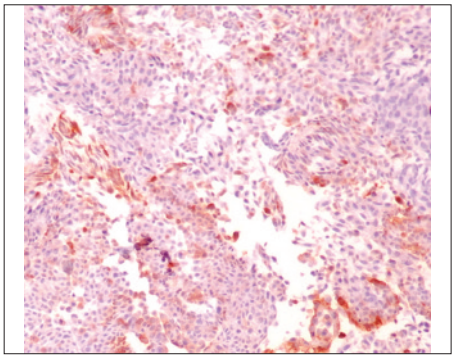

Figure 2: Low grade urothelial carcinoma CD10 immunostaining (+1).

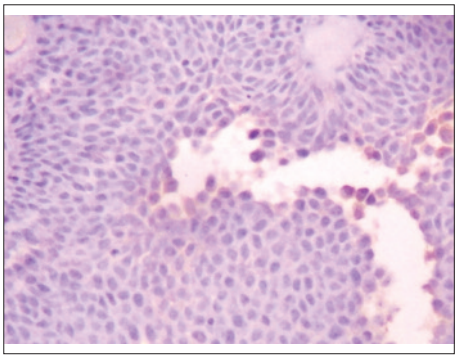

Figure 3: Low grade urothelial carcinoma with negative CD10 immunostaining.

Table I: Comparison of tumor characteristics in different age groups.

Tumor characteristics	Population characteristics (age based groups)			p-value
	Total n=50	Group 1 n=40 (> 50 years)	Group 2 n=10 (< 50 years)	
Grade				
Papillary neoplasm of low malignant potential	5 (10%)	1 (2.5%)	4 (40%)	< 0.001
Low grade papillary urothelial carcinoma	22 (44 %)	18 (45 %)	4 (40%)	
High grade papillary urothelial carcinoma	23 (46 %)	21 (52.5%)	2 (20%)	
Stage				
pTa	9 (18%)	4 (10%)	5 (50%)	0.01
pT1	19 (38%)	16 (40 %)	3 (30%)	
pT2	22 (44%)	20 (50 %)	2 (20%)	
CD10 intensity expression				
Negative	10 (20%)	5 (12.5%)	5 (50%)	0.027
+1	20 (40%)	17 (42.5%)	3 (30%)	
+2	20 (40%)	18 (45%)	2 (20%)	

Table II: Relationship of tumor grades with CD 10.

CD10 expression	Grade			p-value
	Papillary neoplasm of low malignant potential n=5 (%)	Low grade papillary urothelial carcinoma n=22 (%)	High grade papillary urothelial carcinoma n=23 (%)	
Negative	5 (100%)	5 (22.7%)	0 (0%)	
+1	0 (0%)	17 (77.3%)	3 (13.04%)	< 0.001
+2	0 (0%)	0 (0%)	20 (86.95%)	

Table III: Relationship of tumor stage with CD 10.

CD10 expression	Stage			p-value
	pTa n=9 (%)	pT1 n=19 (%)	pT2 n=22 (%)	
Negative	9 (100%)	1 (5.3%)	0 (0%)	
+1	0 (0%)	17 (89.9%)	3 (13.6%)	< 0.001
+2	0 (0%)	1 (5.3%)	19 (86.4%)	

urothelial carcinoma i.e. 20 (86.95%) had more than 50% staining level while 3 (13.04%) showed less than 50% staining level. The difference between different grade of tumors and their CD 10 expression ability was statistically significant ($p < 0.001$, Table II, Figure 1 - 3).

When tumors of different stages were analyzed for expression of CD 10, it revealed that none of the tumors of stage pTa was positive for CD 10 expression. Out of all patients with stage pT 1 tumor, 1 (5.3%) were CD 10 negative and 17 (89.5 %) were CD 10 positive with +1 staining i.e. 5 - 50% staining and 1 (5.3%) had +2 staining i.e. more then 50% expression. Out of all patients with stage pT 2 no tumor was CD 10 negative, 3 (13.6%) cases were CD 10 positive with staining +1 and 19 (84.4 %) patients with stage pT 2 tumor had stained positive with CD 10 with +2 staining. The difference between different stages of tumors and their CD 10 expression ability was statistically significant ($p < 0.001$, Table III).

DISCUSSION

The recurrence of urothelial carcinoma is a major problem and despite improvement in treatment options the recurrence following resection in tumor with stage Ta and T1 is as high as 80%.[4] There are different molecular markers which are used for prognostic studies. In this study, CD10 IHC expression was demonstrated in all high grade urothelial carcinomas of the bladder. CD10

staining of the malignant cells revealed a strong correlation not only with histologic grade but also with pathologic stage. Moreover, percentage of CD10 staining appeared to increase with higher grade; 20 of the 23 high grade carcinomas showed 2+ reaction. Vast majority of the low-grade carcinomas had 1+ staining pattern. The same was true for invasive tumors: 2+ reaction was seen in 19 of 22 pT2 carcinomas. There was a significant correlation between both CD10 immunohistochemical (expression and scoring) and the (WHO 2004) grade of urothelial carcinoma, similar results were obtained by Bahadir *et al*.,[5] Kandemir *et al*.[13] and Mohammad *et al*.[14]

Koiso *et al*. were among the pioneers who studied CD 10 expression in bladder.[15] They found that both enzyme activity and IHC expression were higher in superficial cancers than invasive cancers and normal urothelium. They concluded that CD10 was expressed at a certain stage of differentiation in the course of neoplastic process. The CD 10 is not expressed in non-neoplastic urothelium was shown after the development of CD10 monoclonal antibody appropriate for paraffin-embedded tissues. Later, Chu and Arber showed positive cytoplasmic staining in 13 of 24 (54%) urothelial carcinomas, while there was no reaction in non-neoplastic tissues.[16] However, they did not investigate any correlation of low and high grade or stage with CD 10 expression. These results suggest that neoplastic tissues rather than non-neoplastic epithelium have a propensity for CD10 expression.

Murali *et al*. demonstrated CD10 expression in 80% of invasive carcinomas and also proved that the staining intensity for the high grade group (including invasive carcinoma, high-grade papillary urothelial carcinoma, and carcinoma *in situ*) was statistically higher than that of the low-grade group (including low-grade papillary urothelial carcinoma, papillary urothelial neoplasm of low malignant potential and normal urothelium).[17] In another study by Bircan *et al.* demonstrated CD 10 staining in 34 of 79 (43%) urothelial carcinomas including only one case of non-neoplastic epithelium.[18] They found an inverse correlation between CD10 expression and tumor stage, but no association with histologic grade or staining score was detected. The authors proposed that the higher level of CD10 expression in non-invasive carcinomas appears to inhibit cell invasion.

Abdou *et al*. demonstrated CD10 expression in urothelial carcinoma and its correlation with parameters like advanced stage, tumor size, and shorter mean survival but not with grade.[10] The authors suggested that CD10 appears to be associated with tumor progression and that it could play a pivotal role in bladder cancer pathogenesis. Recently, Bahadir *et al*. in his study assessed CD 10 expression in urothelial carcinoma in urinary bladder and indicated strong correlation with high grade and stage of tumor and its association with tumor progression in bladder cancer pathogenesis.

Apparently, this study, in which CD10 staining increased with grade and stage, bears some similarities with and shows some differences from these previous reports. There may be several possibilities about the role of CD10 in urothelial tumorigenesis. First, CD10 is a cell surface metalloprotease and one can easily postulate that CD10 expressing tumors have the capacity to create a microenvironment that facilitates cancer cell invasion and metastasis.[19] This appears to be the most likely explanation for the significant correlation of CD10 with the grade and stage in this study. If this is the case, it may also be speculated that since invasive bladder carcinomas most likely originate from high-grade non-invasive lesions rather from low-grade tumors,[20] tumors with high grade and stage seem more likely to express CD10. Another possible mechanism is that the increased IHC CD10 expression with increasing grade and stage may indicate accumulation of mutated, non-functional CD10 rather than its normal counterpart.[17]

In studies of melanomas, CD10 expression strongly correlates with tumor progression and metastasis. In a study by Bilalovic *et al*., CD10 expression was significantly higher in primary melanomas with higher Clark level and larger Breslow thickness.[21] These findings are somewhat parallel to the present results, since Clark level and larger Breslow thickness are two important prognostic determinates associated with metastasis in melanoma and the same is true for tumor grade and stage in bladder carcinoma. CD10 is expressed in stromal tissue of colorectal carcinomas, and in invasive ductal carcinomas of the breast but not in normal tissues, a finding supporting the hypothesis that CD10 may facilitate invasion and metastasis.[19,22] CD 10 is also expressed in renal cell carcinoma and follicular lymphoma.[23] Therefore, it may be said that CD 10 may have a role in neoplastic processes probably by mechanism which are not completely identified.

CONCLUSION

The grade and stage of urothelial carcinoma was higher in older age. The CD 10 expression was directly related to grade and stage of tumor and may be associated with tumor progression in bladder cancer pathogenesis. However, exact mechanism of action or biological process through which it regulates neoplastic processes is still not known.

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