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CD10 Expression in various grades and stages of urothelial bladder carcinoma

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Abstract
Objective: To evaluate CD10 expression in urothelial bladder carcinoma and the association of CD10 intensity with grades and stages.
Materials and methods: Thirty consecutive cases of urothelial bladder carcinoma, obtained through transurethral resections, were included in this study. Haematoxylin-eosin (HE) stained sections from each case were evaluated histopathologically according to WHO 2004 grading system. The TNM system was used for pathological staging. CD10 marker was applied and a semi quantitative 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 CD10 expression and age group. P <0.05 was taken as level of significance.
Results: Urothelial carcinoma is more common in males. The male to female ratio was 5:1. The older patients >50 years had higher grades and stages as compared to the younger patients. All cases of high grade urothelial carcinoma showed higher positivity for CD10 expression. Fourteen cases (77%) of high grade urothelial carcinoma were positive with +2 immunostaining while four cases (22%) were positive with +1 staining. Of total nine cases pTa, three cases (33%) were negative and six cases (66%) were positive with +1 staining. Of all the cases of pT1 tumour, 1 case (6.6%) was CD10 negative, 5 cases (33%) were CD10 positive having +1 staining and 9 cases (60%) were CD10 positive having +2 staining. Out of all the patients with stage pT2 no tumour was CD10 negative, 1 case (16.6%) were Cd10 positive with +1 staining and 5 (83.3%) with stage pT2 tumour had staining positive with +2 staining.

Keywords: urothelial carcinoma, histopathological grade and stage, cd10 expression, staining intensity

Introduction

Cancer of the urinary bladder represents the ninth most common cause of cancer worldwide and the 13th most common cause of cancer death [1]. Bladder carcinoma is more common in whites, with male to female ratio of 3:1 and the median age of diagnosis is 68 years [2]. In India the incidence of bladder cancer is 1.6 % with the mortality of about 1.4% and age standardized rate of 1.6/100000 population [3]. Approximately 90% of malignant urinary bladder cancers are transitional cell carcinoma with highest incidence in the 6th and 7th decades of life with man affected more than women [3]. In approximately 75-85% bladder cancer patient, the disease is confined to the mucosa and has a prolonged clinical course with multiple recurrences after local resection without tumor progression [4]. In contrast, a smaller but significant percentage of patients have advanced and muscle infiltrative tumor at the time of diagnosis [5].

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 chemotherapy. Nonetheless, these patients still have a high risk of recurrence following initial resection.[12]. A number of pathologic features have been identified, which are accurate predictors of the clinical course of bladder cancer. The most important are depth of invasion, if any, at presentation, multifocality, a history of prior urothelial tumors, tumor size and grade. Tumor size and grade are not as important an influence on the recurrence rate as tumor multifocality, a prior history of tumors, and depth of invasion at diagnosis [6]. 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.

Pathologic stage is the most important predictor of survival in urothelial carcinoma. Although a 5-year survival rate of approximately 75% is to be expected in a patient with no more than lamina propria invasion at the time of cystectomy, 5-year survival rates of tumors infiltrating muscularis propria of perivesical fat are 50% and 20%, respectively [7] To date few studies have investigated the staining intensity and pattern of CD10 expression in urinary bladder.

CD10 is also known as common acute lymphocytic leukemia antigen(CALLA).[3]The CD10 is a single-chain, 90-110kDa cell surface zinc dependent metalloprotease that inactivates various bioactive neuropeptides [1]. 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 metalloprotease in the stroma, CD10 is also thought to affect invasion and metastatic potential of tumor cells by altering the cellular microenvironment [8].

This was initially discovered on the surface of acute lymphoblastic leukemia cells, and considered to be tumor specific antigen [9].

This marker has a natural endopeptidase activity and is known to regulate biological activities of peptide substrates. There are recent evidence demonstrating a correlation between apoptosis and CD10 expression [10, 11] The present

study was done to evaluate CD10 immuno histo chemical expression in urothelial carcinoma of the urinary bladder and its association with various histopathological grades and stages, thus contributing as a prognostic factor.

Meterials and Methods

A cross sectional study was conducted with thirty consecutive cases of papillary urothelial neoplasm of the urinary bladder over the period of one year. Transurethral resections of bladder tumor were received in the department of pathology, Vivekananda Institute of Medical Sciences, Kolkata. Hematoxylin and eosin stained sections from formalin fixed paraffin embedded specimen were evaluated histo pathologically. All tumors of papillary neoplasm were included in the study and tumors are sub-classified according to WHO (2004) grading system. TNM system was used for pathological staging for non-invasive and invasive papillary urothelial neoplasm. All benign lesions and poorly fixed specimens were excluded from the study. Immuno histo chemical study for CD10 was performed on paraffin embedded formalin fixed sections as per standard procedure. Additional sections from renal tissue were also used as positive control. Staining of cell membrane/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 tumor cells were positive); 1+ (5-50% of tumor cells were positive); and 2+ (>50% of tumor cells were positive [7]. Statistical analysis was performed using Statistical package for social sciences (SPSS) version 17. Quantities variables like grades and stages of urothelial carcinoma along with CD10 expression were calculated in terms of frequency and percentages. Fischer's exact test was used to compare grades, stages of urothelial carcinoma with CD10 expression and age groups. P< 0.05 was taken as level of significance.

From this study of CD10 expression in urothelial carcinoma of urinary bladder we will be able to know how it is associated with different grades and stages of urothelial carcinoma and that CD10 may be an independent predictor of tumor progression in urothelial cancer pathogenesis.

Results and Analysis

Thirty cases of urothelial carcinoma were included in this study. Majority of patients i.e. 27 (90%) were more than 50 years old while 3 (10%) were younger than 50 years (Table-1). Predominant population was male 25 (83.33%) and only 5 (16.66%) females. Number of older patients suffering from high grade and stage urothelial carcinoma was higher. Among older patients16 (59.2%) has high grade papillary urothelial carcinoma, and 11(40.7%) has low grade papillary urothelial carcinoma. In young patients 1 (33%) had low grade papillary urothelial carcinoma and 2 (66%) had high grade papillary urothelial carcinoma. The relationship of age group with tumor grades was statistically insignificant (p >>>0.05), (Table 1). This statistical insignificance is probably due to small sample size which may not reflect the exact picture, or it may also be true that grade and stage has no relation with age.

Table 1

Tumour Characteristics Histological Grade	Population Characteristics (Age Based Group)			p-value >>>0.05
	Total n=30	Group-1 Age (<50yrs) n1=3	Group-2 Age(>50yrs) n2=27	
Low	12(40%)	1(33%)	11(40.7%)	
High	18(60%)	2(66%)	16(59.2%)	

On histological examination 9 (30%) had non-invasive tumor (pTa), further 15 (50%) had invasion restricted to lamina pro Pria (pT1) and remaining 6 (20%) had advanced stage tumor invading lamina propria as well as muscles (pT2) (Table-2).

Table 2

Tumour Characteristics TNM staging	Population Characteristics (Age Based Group)			p-value >>> 0.05
	Total n=30	Group-1 Age(<50yrs) n1=3	Group-2 Age(>50yrs) n2=27	
PTa	9(30%)	1(33%)	8(29.6%)	
PT1	15(50%)	1(33%)	14(51.8%)	
PT2	6(20%)	1(33%)	5(18.51%)	

In the present study age based distribution of different stages showed that in the higher (>50 year) age group, out of total 27 cases, 8(29.6%) was in pTa, 14 (51.8%) was in pT1 and 5 (18.51%) was in pT2 staging group. (Table-2). In lower (<50 year) age group, out of total 3 cases, 1 (33%) was in pTa, 1 (33%) was in pT1 and 1 (33%) was in pT2 staging group (Table-2). The age based distribution of different stages of tumor is statistically insignificant (p >>>0.05). So this study reveals that there is no age specific relationship with different grades and stages of tumor. This statistical insignificance is probably due to small sample size which may not reflect the exact picture, or it may also be true that grade and Stage has no Relation with age.

When tumors of different grades were analyzed for expression of CD10, it revealed that of all the patients with low grade papillary urothelial carcinoma, 4 (33%) were CD10 negative and 8 (66%) were CD10 positive with level one (+1) expression i.e. 5-50%. Most of the patients with high grade urothelial carcinoma i.e. 14 (77%) had more than 50% (+2) staining level while 4 (22%) showed less than 50% (+1) staining level. None of the tumors of high grade papillary urothelial carcinoma show negative expression for CD10. The difference between different grades of tumor and their CD10 expression ability was statistically significant (<<<0.05, Table-3)

Table 3

Tumour Characteristics IHC CD 10 Expression	Tumour Characteristics Histological Grade		
	Low grade Group-1 n1=12	High grade Group-2 n2=18	
Negative	4(33%)	0(0%)	p-value <<< 0.05
+1	8(66%)	4(22%)	
+2	0(0%)	14(77%)	

When tumors of different stages were analyzed for expression of CD10, it revealed that out of 9 cases pTa, 3 (33%) cases showed negative expression for CD10, 6 (66%) were CD10 positive with +1 staining i.e. 5-50% staining and none of the patient of pTa had expressed +2 staining i.e. more than 50%expression. Out of total 15 patients with pT1 tumor, 1 (6.6%0) were CD10 negative, 5 (33%) were Cd10 positive with +1 staining i.e. 5-50% staining and 9 (60%) had +2 staining i.e. more than 50% expression. Out of total 6 patients with stage pT2 no tumor was CD10 negative, 1 (16.6%) case was CD10 positive with staining +1 and 5 (83.3%) patients with stage pT2 tumor had stained positive with CD10 with +2 staining. The difference between different stages of tumors and CD10 expression ability was statistically significant (p=0.01, Table- 4).

Table 4

Tumour Characteristics IHC CD 10 Expression	Tumour Characteristics TNM Staging			
	PTa Group-1 n1=9	PT1 Group-2 n2=15	PT2 Group-3 n3=6	
Negative	3(33%)	1(6.6%)	0(0%)	p-value 0.010
+1	6(66%)	5(33%)	1(16.6%)	
+2	0(0%)	9(60%)	5(83.3%)	

Discussion

Cancer of the urinary bladder represents the ninth most common cause of cancer worldwide and the 13th most common cause of cancer death [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 of diagnosis is 68 years[2]. In India the incidence of bladder cancer is 1.6 % with the mortality of about 1.4% and age standardized rate of 1.6/100000 population [3]. Approximately 90% of malignant urinary bladder cancers are transitional cell carcinoma with highest incidence in the 6th and 7th decades of life with man affected more than women[3]. In approximately 75-85% bladder cancer patient, the disease is confined to the mucosa and has a prolonged clinical course with multiple recurrences after local resection without tumor progression [4]. The recurrence of urothelial carcinoma is a major problem and despite improvement in treatment options the recurrence following resection in tumor with stage pTa and pT1 is as high as 80%. [12]There are different molecular markers which are used for prognostic studies. In this study, CD10 immuno histo chemical (IHC) expression was demonstrated in all high grades urothelial carcinoma of the urinary bladder.CD10 staining of the malignant cells revealed a strong correlation not only with histologic grade but also with pathologic stage. Percentage of CD10 staining appeared to increase with higher grade. There was a significant correlation between both CD10 immuno histo chemical (expression and scoring) and the grade of urothelial carcinoma. Similar results were obtained by Bahadir *et al*, Kandemir *et al* and Mohammad *et al* [1, 15, 20]. To date few studies have investigated the staining intensity and pattern of CD10 expression in urinary bladder.

In the present study total 30 (thirty) consecutive cases of urothelial carcinoma of urinary bladder were studied for one and half years. Inthe present study it is found that male to female ratio is 5:1. The patients age ranged from 35 to 93 years (mean ±SD 65±12.143).

There are several possibilities about the role of CD10 in urothelial tumorigenesis.CD10 is also known as common acute lymphocytic leukemic antigen (CALLA).[3]CD10 is single chain 90-110 kDa single chain antigenic molecular marker which is a cell surface zinc dependent metallo protease and one can easily postulate that CD10 expressing tumors have the capacity to create a microenvironment that facilitates cancer cells invasion and metastasis[3, 11]. These appear to be likely explanation for the significant correlation of CD10 with grade and stage. It may also be speculated that invasive bladder carcinoma most likely originate from high grade noninvasive lesion rather from low grade tumor[13]. Another possible mechanism is that the increased IHC CD10 expression with increasing grade and stage may indicate accumulation of mutate nonfunctional CD10 rather than its normal counterpart [14].

Atique M, Abbasi MS, *et al* [2]. were among the pioneer who studied CD10 expression in urothelial carcinoma of urinary bladder made with fifty consecutive cases. Their study revealed that 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 CD10. Most of the patients with high grade 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 CD10 expression ability was statistically significant [2]. Mrurali *et al* demonstrated CD10 expression in 80% of urothelial carcinomas and also proved that staining intensity for high grade group was statistically higher than that of low grade group [14] Burak Bahadir, Kemal Behzatoglu *et al* [1]made a study with 371 cases of urothelial bladder carcinomas. Their study shows the overall CD10 expression according to the histologic grade and pathologic stage. 157 of the 371 cases (42.3%) showed CD10 immuno staining while 214 cases (57.7%) were negative for CD10. C010 immuno histo chemical reaction was higher with higher histologic grade ($p < 0.0001$) [1].

Urothelial carcinomas reveal CD10 expression (focal in low grade and diffuse in high grade), unlike cases with urothelial

dysplasia in which CD10 expression is negative [18] Furthermore, CD10 expression significantly correlates with certain parameters in urothelial carcinomas, such as advanced stage, tumor size, and shorter survival [13, 19] In addition, CD10 expressing cells are supposed to have increased potential for invasion and metastasis.

Ahmed Salahaldden Mohammed, Husan Hossen Ali, *et al* made a study with forty-nine cases of urothelial carcinoma. Their study shows that the staining pattern and intensity of CD10 for different histopathological grade of tumor. It shows statistical significantly greater score of CD10 staining pattern and intensity with increase grade of urothelial carcinoma [15].

Koiso *et al* were among the pioneer who studied CD10 expression in bladder. They concluded that CD10 expressed at a certain stage of differentiation in the course of neoplastic process [16]. Neoplastic tissue rather than non-neoplastic epithelium has a propensity for CD10 expression.

Chu and Arber showed positive cytoplasmic staining in 13 of 24 (54%) urothelial carcinoma, while there was no reaction in non-neoplastic tissue [17].

H. Popov, P. Ghenev. *et al*. also conducted a study about expression 0f CD10 as a prognostic and predictive factor in urothelial carcinoma [21].

The absence of IHC CD10 expression in non-neoplastic urothelium was shown after the development of CD10 monoclonal antibody appropriate for paraffin embedded tissues.[22]Kandemir *et al* suggested that CD10 expression in high grade urothelial carcinoma may be an outcome of genetic changes causing wild type or mutant type CD10 antigenic expression in the high grade carcinomas [20].

In the present study, when tumors of different grades were analyzed for expression of CD10, it revealed that of all the patients with low grade papillary urothelial carcinoma, 4 (33%) were CD10 negative and 8 (66%) were CD10 positive with level one (+1) expression i.e. 5-50%. Most of the patients with high grade urothelial carcinoma i.e. 14 (77%) had more than 50% (+2) staining level while 4 (22%) showed less than 50% (+1) staining level. None of the tumors of high grade papillary urothelial carcinoma show negative expression for CD10. The difference between different grades of tumor and their CD10 expression ability was statistically significant (p<<<0.05, Table 4). So this study matches with the observation that greater the grade of urothelial carcinoma, greater the expression of intensity for CD10 immuno histo chemical marker and lower the grade, lower the expression of intensity for the same marker (p<<<0.05) In this present study we notice when tumors of different stages were analyzed for expression of CD10, it revealed that out of 9 cases pTa, 3 (33%) cases showed negative expression for CD10, 6 (66%) were CD10 positive with +1 staining i.e. 5-50% staining and none of the patient of pTa had expressed +2 staining i.e. more than 50%expression. Out of total 15 patients with pT1 tumor, 1 (6.6%0) were CD10 negative, 5 (33%) were CD10 positive with +1 staining i.e. 5-50% staining and 9 (60%) had +2 staining i.e. more than 50% expression. Out of total 6 patients with stage pT2 no tumor was CD10 negative, 1 (16.6%) case was CD10 positive with staining +1 and 5 (83.3%) patients with stage pT2 tumor had stained positive with CD10 with +2 staining. The difference between different stages of tumors and CD10 expression ability was statistically significant (p=0.01, Table 5).

Fig 1: Papillary urothelial carcinoma Low Grade H & E (20 X).

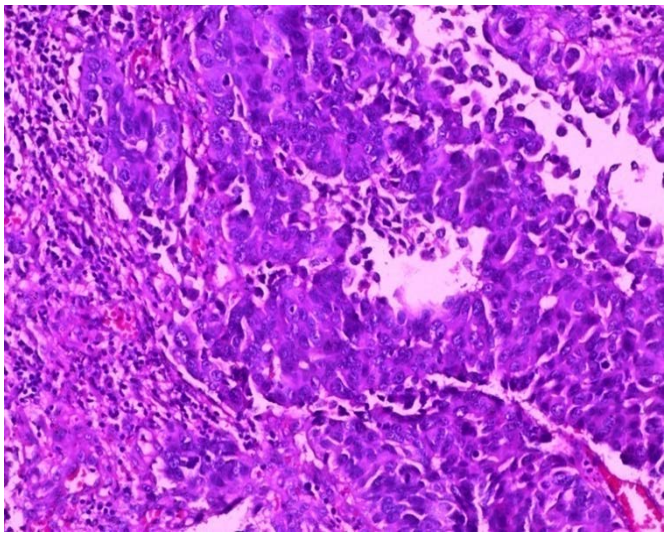

Fig 2: Papillary urothelial carcinoma High grade H & E (20 X).

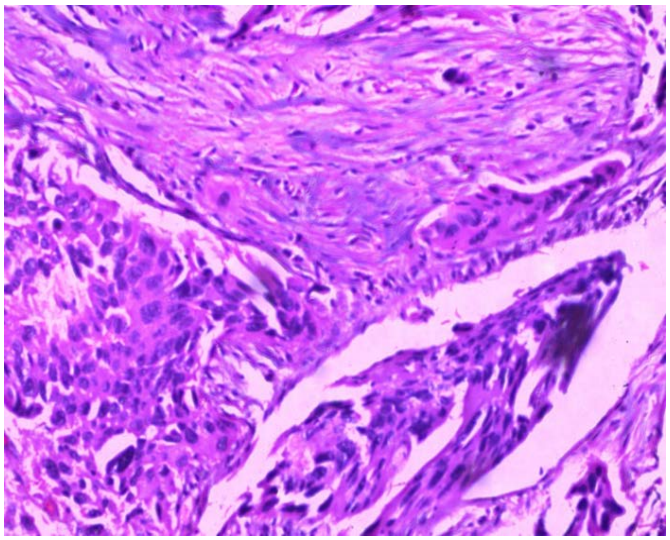

Fig 3: Urothelial carcinoma pT1 H & E (20 X).

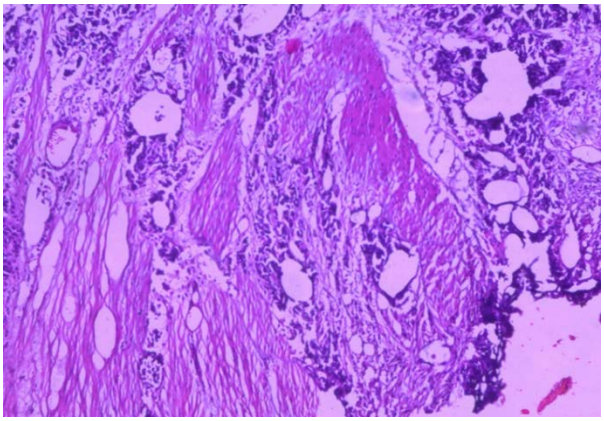

Fig 4: Urothelial carcinoma pT2 H &E (20 X).

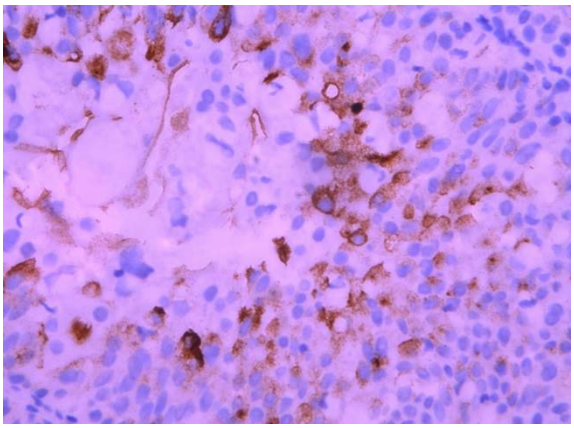

Fig 5: Papillary urothelial carcinoma Low grade CD10 expression +1 (20 X).

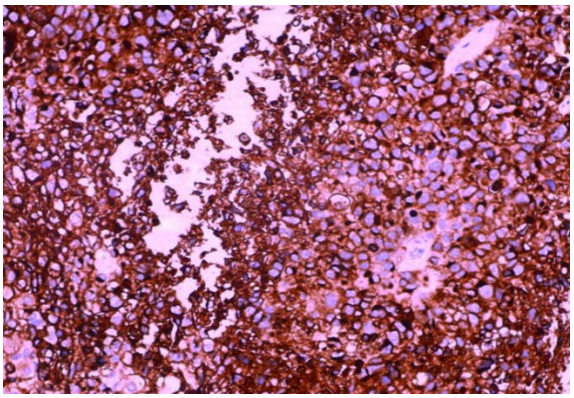

Fig 6: Papillary urothelial carcinoma high Grade CD10 Expression +2 (20 X)

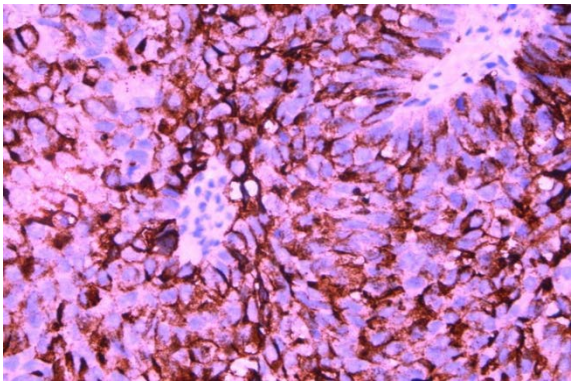

Fig 7: High grade urothelial carcinoma high grade CD10 Expression +2 (20 X)

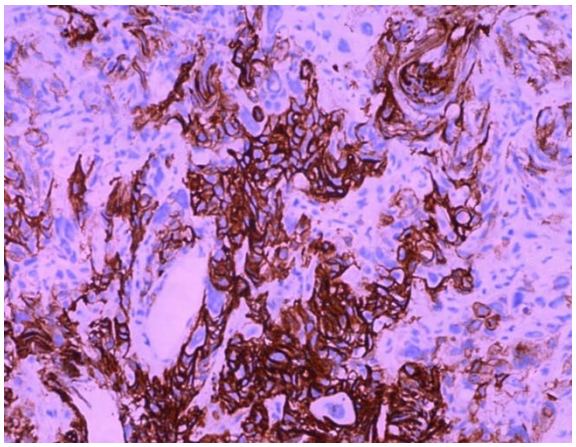

Fig 8: Papillary urothelial carcinoma High grade pT1 CD10 Expression +2 (20 X)

Conclusion

This study concludes that there is significant association between staining intensity and score of CD10 immuno-expression with grades and stages of urothelial carcinoma of the urinary bladder and may be associated with tumor progression in bladder cancer pathogenesis. The study also reveals that urothelial carcinoma has high incidence in male than female. No correlation of grades and stages of urothelial carcinoma with age have been established in this study.

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