International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Research Article

UTILITY OF CD24 IMMUNOHISTOCHEMICAL EXPRESSION IN UROTHELIAL CARCINOMA OF BLADDER

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Received: 02-09-2025
Accepted: 20-09-2025
Available online: 13-10-2025

ABSTRACT

Objective: To study utility of CD24 immunohistochemical expression in urothelial carcinoma in association with tumor grade and stage.
Materials and methods: A cross sectional study was conducted for 16 months at a tertiary care centre on 52 TURBT specimen primarily diagnosed on HE as urothelial carcinoma. Selected block was subjected to CD24 immunohistochemistry and expression of the marker was semi quantitatively scored as low (score 0, 1) and high expression (score 2, 3) based on intensity of cytoplasmic staining. This expression was studied for its association with clinicopathological parameters.
Results: In normal urothelium, CD24 was localized in the luminal basal layer with minimal intensity. Expression of CD24 is upregulated in urothelial carcinoma and high expression (score 2,3) was correlated with high grade, invasive tumor and tumor stage ($p < 0.01$). Invasive high grade urothelial carcinomas show high expression of CD24.
Conclusion: CD24 was overexpressed in significant number of high grade invasive urothelial carcinoma which indicate CD24 as marker of assessing stromal invasion and high tumor grade. This make CD24 not only as prognostic marker but also as theranostic marker for targeted therapy in tumor showing high CD24 expression.

Keywords: *Invasive urothelial carcinoma, immunohistochemistry, high grade, CD24.*

INTRODUCTION

Bladder carcinoma (BC) ranks as the 10th most frequently diagnosed cancer worldwide (1). Urothelial carcinoma constitutes the predominant type, making up 90%, followed by squamous cell carcinoma, adenocarcinoma, and small cell carcinoma. It represents a major source of morbidity and mortality, exhibiting the highest fatality rates. In 2020, the incidence and mortality rates for urothelial carcinoma (UC) were approximately 573,000 new cases and 213,000 deaths, respectively (2).

Urothelial carcinoma develops through unique molecular pathways that parallel histopathological classification. Histological characteristics remain the gold standard for classifying and diagnosing urothelial tract tumours (3). Tumor grading and staging are the most important prognostic markers along with the useful information provided by immunohistochemical and molecular markers in evaluation and treatment of urothelial carcinomas. A multimodality therapy for urothelial carcinoma include TURBT (Transurethral resection of bladder tumor) followed by chemo radiotherapy or neo adjuvant chemotherapy followed by TURBT have shown good long term survival (4) when compared to single modality treatment Immunohistochemical markers serve as additional criteria in risk stratification of

patients with non-invasive urothelial carcinoma (5). Biomarkers that are overexpressed during cancer progression are of special interest as they can be used for both prediction of outcome as well serve as potential target in adjuvant cancer therapy.

Cluster differentiation 24 (CD24), is small, heavily glycosylated mucin-like cell surface protein that is attached to the cell membrane by a GPI (glycosylphosphatidylinositol) anchor. It was originally described as a B-cell specific marker expressed in the early stages of B-cell development (6), but studies have shown that CD24 is expressed in many human malignancies including breast, ovarian, colorectal, pancreatic, prostate, bladder cancer and higher levels of expression are associated with increased aggressive course and worse prognosis (7). CD24 acts as a specific ligand for P-selectin, an adhesion molecule expressed by endothelial cells and platelets. There are only few studies describing the overexpression of CD24 in bladder urothelial carcinomas and have shown strong CD24 immunoreactivity with shortened disease-free survival. The aim of this study is to evaluate and validate the immunohistochemical expression of CD24 in bladder urothelial carcinomas and correlate with possible associations with clinicopathologic parameters which may aid in providing useful additional information regarding aggressive tumor behaviour, tumor invasion, staging and management of invasive urothelial carcinoma.

MATERIALS AND METHOD

A total of 52 patients with bladder urothelial carcinomas were included in the study who underwent TURBT at tertiary cancer centre in Southern India between March 2021 to June 2022. Patients with histopathological diagnosis of primary Urothelial carcinoma, who did not receive any prior chemotherapy or radiotherapy before TURBT, availability of clinical data and paraffin-embedded tissue blocks were included. Non-neoplastic, inflammatory lesions and carcinomas other than urothelial carcinoma and secondary tumours were excluded from the study. The study was conducted after obtaining approval from Institutional Ethics Committee (IEC).

This is single institute cross sectional study done for16 months in Department of Pathology, aimed to study the utility of CD24 expression in morphologically diagnosed cases of Urothelial carcinoma. The patients included in the study were followed till the study period. Paraffin embedded tissue blocks were prepared, micro sections were obtained and Haematoxylin and Eosin (H&E) staining was performed. All the tissue samples were histologically evaluated and cases with morphological diagnosis of urothelial carcinomas were graded and staged according to the TNM 2016 classification (8). Two normal control tissue sections were run for CD24; from one normal urothelium in cystectomy specimen for non-neoplastic condition and one normal colon tissue.

For Immunohistochemistry 3µ sections on APES (3-3Amino Propyl Triethoxy Silane) coated slides and incubated overnight at 37°c. Sections were deparaffinised in xylene for 5-10 min and rehydrated through graded series of alcohol. Endogenous peroxidase activity was quenched by incubating in 3% Hydrogen peroxide for 10 minutes. Enzymatic digestion done with trypsin and antigen retrieval for recovery of antigenicity was done by heat with Pressure cooker method in Tris-EDTA pH 9.0. Sections were **w**ashed in Tris buffer pH 7.6 and power Block Reagent (buffered 3% casein solution with Sodium azide) for 10 minutes: A highly effective universal protein blocking reagent to block the non-specific protein-protein interactions and sections were incubated with primary antibody anti-CD24 antibody in 1: 50 dilution for 30 minutes at room temperature in moist chamber to prevent drying. After 3 changes of Tris buffer for 5 minutes each, sections were incubated with Super Enhancer Reagent for 20 minutes at room temperature. After wash in TRIS buffer pH 7.6, Poly-HRP (Horse Radish Peroxidase) Reagent (which is conjugated to anti-rabbit secondary antibody) and sections incubated for 20minute at room temperature. For chromogen, substrate-chromogen mixture (1 ml of Substrate Buffer + 1 drop of Liquid Di amino Benzidine (DAB) chromogen solution), was added to tissue section and incubated for 10-15 minutes at room temperature. Sections were counterstained with Harris hematoxylin. Normal colonic tissue section was used as positive control.

The intensity of CD24 staining was semi quantitatively scored as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). Cases in which the percentage of positive cells is less than 10% with weak intensity were considered as negative (score 0). This pattern was seen in normal urothelium with only weak and focal apical cytoplasmic staining. In sections containing more than 10% of the area staining positive, the sections were scored by intensity as mild, moderate and intense (score 1, 2, and 3) (Fig 2). Each lesion was examined and scored separately by 2 pathologists (B.D. and N.R.G). Cases with discrepant scores were discussed with senior pathologist (A.K.P) till a uniform decision was reached. The final evaluation assigned samples to 2 groups: Group I (low-level expression) for sections with a score of 0 or 1, and Group II (high-level expression) for sections with a score of 2 or 3 (Fig 2).

Statistical analysis was done using the software Statistical Package for social Sciences (SPSS) 22.0 version. All categorical variables were expressed as counts and percentages. Chi-square test and Student t test was used to evaluate the association between clinicopathological parameters and CD 24 IHC marker expression. Probability (p) value <0.05 were considered as statistically significant.

RESULTS

The age of patient in the study ranged from 42 to 82 years with a mean age of 64 years. There were 43 male (82.7%) and 9 female patients (17.3%) with a male to female ratio 4.7:1. There were 35 (67.3%) patients above 60 years of age and there were 17 (32.7%) patients below 60 years of age. Majority of the cases were from 6th decade followed by 7th decade. 34 patients (65.4%) patients had history of tobacco use either in the form of smoking or tobacco chewing
Among the 52 cases, the most common site of tumour in the urinary bladder was the posterior wall (15 cases, 28.8%), followed by left lateral wall (11 cases, 21.1%), anterior wall (10 cases, 19.2%), right lateral wall (10 cases, 19.2%) and diffuse involvement (6 cases, 11.5%).

The grading of urothelial carcinomas was done according to international society of urological pathology 2016 classification (8); 37 (71.2%) patients were categorised as high-grade urothelial carcinomas while only 15 patients (28.8%) were classified as low grade. 73% (n=38) of urothelial carcinomas were invasive and 27% (n=14) were diagnosed as non-invasive carcinomas (Fig 1). The results are summarised in the table 1.
In the present study, among the 38 cases with invasive urothelial carcinoma, high grade morphology was seen in 34 cases (89.5%) and low grade was seen in 4 cases (10.5%). Among the 14 non-invasive urothelial carcinomas, only 3 cases were high grade morphology (21.4%) and low grade was seen in 11 cases (78.6%). The association between the invasiveness of the tumour and morphological grade was significant with p-value of <0.01.

Among the 52 cases, included in the study majority belonged to stage T1 (19 cases, 36%) followed by stage T2b (17 cases, 33%), followed by stage Ta (14 cases, 27%) and the least number of cases in stage T2a (2 cases, 4%).
Lymphovascular invasion was seen in 19 (36.5%) and perineural invasion was seen only in 9 cases (17.3%) (Fig 3).
CD24 immunostaining showed a weak immunoreactivity with distinct well defined apical cytolplasmic/membrane localisation in the normal urothelium as well as non-neoplastic urothelium adjacent to the urothelial carcinomas. The 52 cases studied; 12 cases (23%) showed low expression of CD24 and 40 cases (77%) showed high expression of CD24 (Fig 2).

Total cases included in the study were divided into two groups based on age, ≤60 years (17 cases, 33%) and >60 years (35 cases, 67%). Association between this age division and CD 24 expression was studied. CD 24 high expression was significantly associated with elderly age group (p<0.05) as majority of them were high grade and invasive.
Of 52 total cases of urothelial carcinomas, a high expression of CD 24 was detected in 40 cases (77%). In morphologically high-grade urothelial carcinoma (37 cases, 71.2%), CD 24 high expression is seen in 35 cases (94.6%) and low expression in 2 cases (5.4%). In low grade urothelial carcinoma (15 cases, 28.8%), 5 cases showed high CD 24 expression and 10 cases showed low CD24 expression. CD24 high expression was significantly associated with high tumour grade with p-value <0.01

In 38 cases of invasive urothelial carcinoma, the CD24 expression was high in 35 cases (92.1%) and low in 3 cases (7.9%). Among the 14 cases of non-invasive urothelial carcinoma, CD24 expression was high in 5 cases (35.7%) and low in 9 cases (64.3%). CD24 expression was significantly high in infiltrating urothelial carcinoma. with p-value <0.01
2 cases belonging to stage T2a showed high expression of CD24 (100%). Among 17 cases of stage T2b, 16 cases (94.1%) showed high CD24 expression. CD24 high expression was seen in 17 cases (89.5%) in stage T1 and 5 cases (35.7%) in stage Ta. High expression of CD24 was significantly associated with high tumour stage with the p-value of <0.01. The tumors showed significant high CD24 expression in morphological high grade and high tumor stage with 89.5%, 100%, 94.1% expression in T1, T2a, T2b respectively Table 1.

Lymphovascular invasion was seen in 19 cases (36.5%) of which 18 cases (94.7%) showed high CD24 expression. Among the 33 cases (63.5%) negative for lymphovascular invasion, 22 cases (66.7%) showed high CD 24 expression and 11 cases (33.3%) showed low CD24 expression. Cases with lymphovascular invasion showed a significant high CD24 expression with p-value of 0.021.

Of the 52 cases, follow-up data were available for 48 patients and 4 patients were lost to follow-up. Of those 48 cases,25 patients survived resulting in survival rate of 52.1% and 23 patients had succumbed to the illness, resulting in mortality rate of 47.9%. Survival rate in cases with low CD24 expression (11 cases, 23.1%) is 90.9% and mortality is 9.1 %. In cases with high CD24 expression (35 cases, 76.9%) survival rate was 40.5% and mortality rate was 59.5%. Hence mortality rate was higher in patients with high CD24 expression which was statistically significant with p-value of 0.003.

Table 1: Comparison of clinico-pathological data with CD24 expression

Parameters (n)	**CD 24 expression**		**p value**
	Low expression n (%)	**High expression n (%)**	
Age ≤60 yrs(17)	8 (47.1)	9 (52.9)	0.004
>60 yrs (35)	4 (11.4)	31 (88.6)	
Total	12(23.1)	40(76.9)	
Gender Male(43)	10 (76.9)	33 (84.6)	0.525
Female(9)	3 (23.1)	6 (15.4)	

Total	13(25)	39(75)	
Morphological Grade			
High (n=37)	2 (5.4)	35 (94.6)	<0.01
Low(n=15)	10 (76.9)	5 (12.8)	
Total	12(23.1)	40(76.9)	
Invasive UC(n=38)	3 (7.9%)	35 (92.1)	<0.01
Non-invasive UC(n=14)	9 (64.3%)	5 (35.7)	
Total	12(23.1)	40(76.9)	
Tumour stage Ta (n=14)	9 (64.3)	5 (35.7)	<0.01
T1(n=19)	2 (10.5)	17 (89.5)	
T2a(n=2)	0 (0)	2 (100)	
T2b(n=17)	1 (5.9)	16 (94.1)	
Total	12(23.1)	40(76.9)	
PNI Present (n=9)	1 (8.3%)	8 (20)	0.349
Not present(n=43)	11 (91.7%)	32 (80)	
Total	12(23.1)	40(76.9)	
LVI Present(n=19)	1 (8.3%)	18 (45%)	0.021
Not present(n=33)	11 (91.7%)	22 (55%)	
Total	12(26.1)	40(76.9)	
Tobacco use Yes (34)	6 (17.6)	28 (82.4)	0.202
No (18)	6 (33.3)	12 (66.7)	
Total	12(26.1)	40(76.9)	
Outcome (n=48)			
Surviving (25)	10 (90.9)	15 (40.5)	0.003
Deceased(23)	1 (9.1)	22 (59.5)	

UC- Urothelial carcinoma; PNI- Perineural invasion; LVI- lymphovascular invasion

Figure legends:

Figure 1: A) Non-invasive papillary urothelial carcinoma- low grade; papillae lined by thickened urothelium with mild atypia and central fibrovascular core; Hematoxylin &Eosin X200. B) Invasive high grade urothelial carcinoma showing nests of neoplastic urothelial cells; Hematoxylin &Eosin, X200. C) Invasive urothelial carcinoma showing muscularis propria invasion; Hematoxylin &Eosin X400.

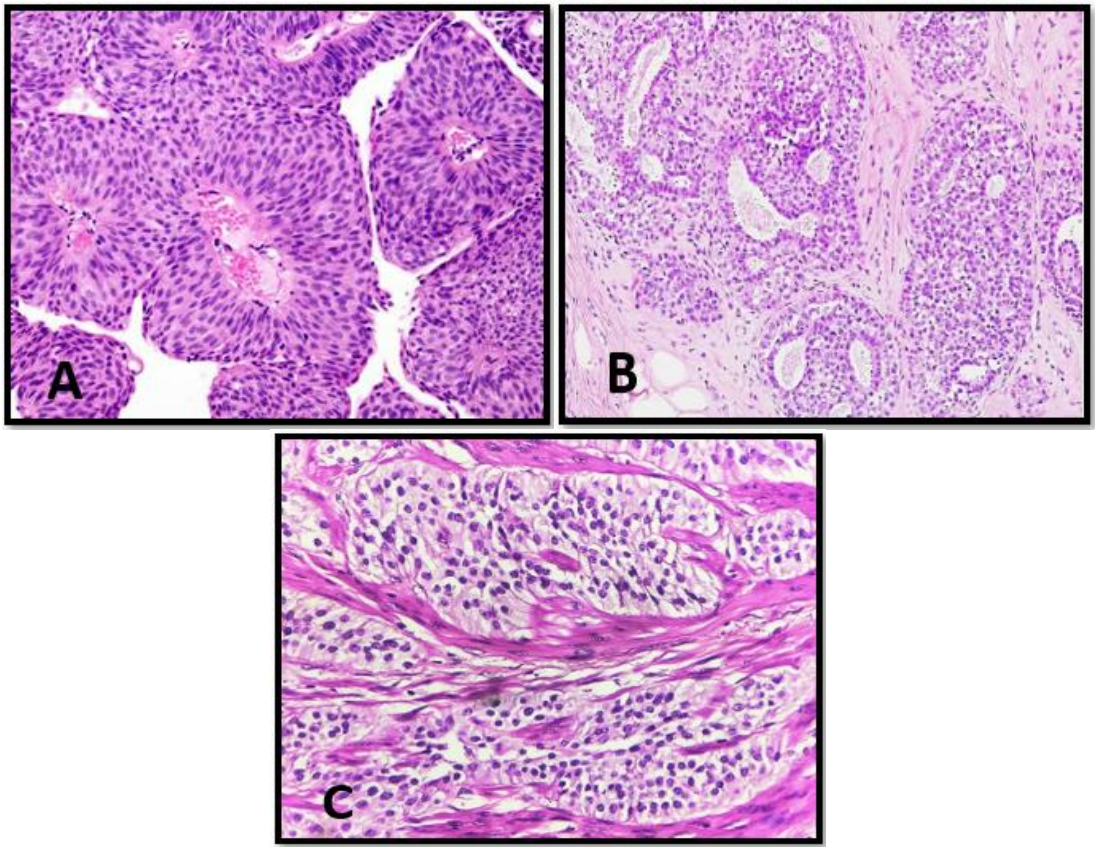


Figure 2: A) Low expression of CD 24 showing weak cytoplasmic staining in Non-invasive urothelial carcinoma Score-1+; CD24 X200. B) Low expression of CD 24 showing diffuse moderate cytoplasmic staining in Non-invasive urothelial carcinoma Score-2+; CD24 X200. C) Diffuse intense cytoplasmic expression of CD 24 (Score-3+) in lesional cells in invasive urothelial carcinoma; CD24 X200.

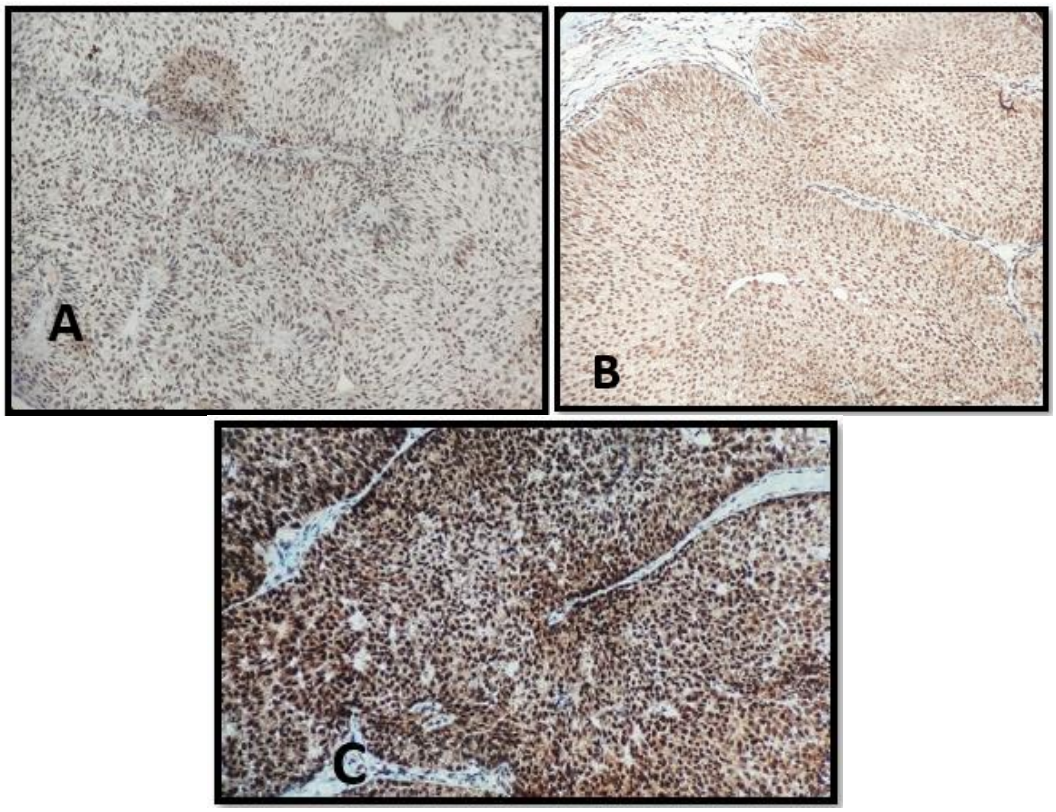
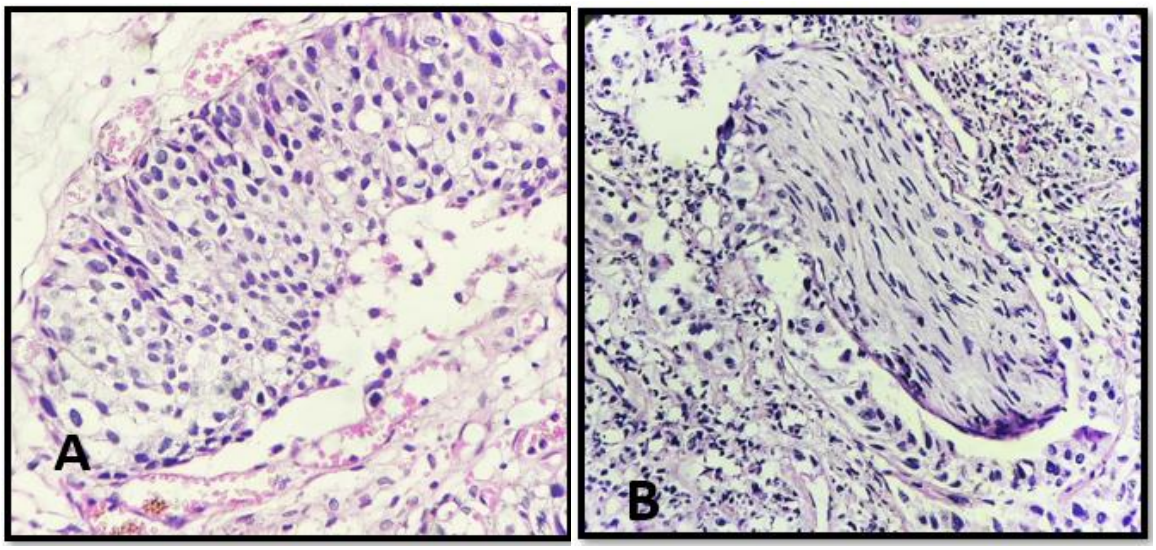


Figure 3: A) Lymphovasacular invasion in invasive urothelial carcinoma; Hematoxylin &Eosin, X400. B) Neoplastic cells encasing the nerve bundle showing perineural invasion; Hematoxylin &Eosin, X400.



DISCUSSION

The present study was done to evaluate the expression of CD24 expression by immunohistochemistry in the bladder urothelial carcinomas and assess its utility as prognostic marker of invasion and grading in TURBT.

In normal bladder urothelium, CD24 was typically localised in the apical cytoplasm with very low expression. In superficial non-invasive tumours the cytoplasmic expression increased and progressed from weak to even moderate

complete cytoplasmic staining. The frequency and intensity of CD24 overexpression increased significantly in invasive carcinomas as well as high grade urothelial carcinomas. The polarized apical expression of CD24 expressed in normal urothelium switched to diffuse and strong complete cytoplasmic positivity with higher grade and invasive tumors. Invasive cancer cells lost the apical polarity of CD24 staining and expressed nonpolarized complete and intense cytoplasmic staining. Increased expression CD24 can be considered as a reliable marker of tumor progress with invasion and higher grade. This finding is in concordance with other similar studies. CD24 is one of the differentially expressed genes in invasive urothelial carcinoma which was consistent with our immunohistochemical expression. Cytoplasmic expression of CD24 could probably reflect an overproduction of the protein or a disturbance in protein distribution or degradation within the tumor cell. Normal cells are organised and maintain the apical polarity. The loss of cellular polarity is a characteristic feature of neoplasia. The molecular sequence leading to loss of polarity and invasiveness of cancer cells is not completely studied yet, but there is a strong correlation between the loss of apical localization of CD24 and the stromal invasion of tumor cells in our study which is similar to the study done by Yoon et al (9). These data suggest that, during tumor progression, the tumor cells may lose membrane polarity, reflected by the nonpolarized expression such IHC markers.

In the current analysis, there were two special scenarios in which the initial histopathological examination reported as Non-invasive high grade urothelial carcinoma, with subsequent CD24 IHC Score-3+, with non-polarised, diffuse cytoplasmic staining prompted for an exhaustive thorough scanning for the foci of stromal infiltration. All the H&E slides were retrieved again reviewed thoroughly and any suspicious foci of invasion and serial deeper sections were taken. Upon extensive full depth scanning in the deeper sections definitive tiny invasive foci was identified. This thereby helped in upstaging the tumour. These scenarios highlight efficiency of increased non-polarised high CD24 expression in determining the possible underlying stromal infiltration especially in TURBT specimens with little underlying stroma. Therefore, the nonpolarized cytoplasmic staining of CD24 may be a novel molecular signature of stromal invasion by carcinomas.

Cytoplasmic CD24 expression is proportionate reflection of protein overproduction or a disruption in protein sorting (9). Kristiansen et al (10) reported that immunostaining using CD24 to study this as individual prognostic marker of survival in non-small cell lung carcinoma. Similar study done by Kristiansen et al (11) done on ovarian cancer that proves CD24 as new independent prognostic marker for patient survival.

In a study by Choi YL et al (12) showed loss of polarity of CD24 expression in advanced ovarian borderline tumor was correlated with advanced stage of the tumors which makes CD24 as novel molecular signature of stromal invasion in carcinomas.

Cancer cells bind to platelets via CD24 mediated binding to P-selectin, which promotes movement of tumor cells into blood stream via lymphovascular channels and increase the chance of metastasis (13) which correlates with our study where significance is noted between overexpression of CD24 and lymphovascular emboli.

In summary, we found that CD24 is overexpressed in bladder urothelial cancers and that cytoplasmic overexpression is a marker of invasive and high-grade tumors. In superficial bladder tumors, in particular, the loss of apical localization of cytoplasmic staining was correlated with stromal invasion. Although the biologic function of CD24 and significance of the changes in expression and cellular localization is not yet clear, our results demonstrate the diagnostic and therapeutic effectiveness of using CD24.

REFERENCES

1. Bray F, Ferlay J, Laversanne M, Brewster DH, Gombe Mbalawa C, Kohler B, et al. Cancer Incidence in Five Continents: Inclusion criteria, highlights from Volume X and the global status of cancer registration. Int J Cancer. 2015 ;137:2060–71.
2. Sung H, Ferlay H, Siegal RL, Laversanne M, Soerjomataram I, Jemal A et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 cancers in 185 countries. CA Cancer L Clin. 2021;71:209-49.
3. Sjödahl G, Eriksson P, Liedberg F, Höglund M. Molecular classification of urothelial carcinoma: global mRNA classification versus tumour-cell phenotype classification. J Pathol. 2017;242(1):113-125.
4. Chiang Y, Cheng JCH, Huang CY, Tsai YC, Lin CC, Hsu CH, et al. A role of multimodality bladder-preserving therapy in patients with muscle-invasive bladder cancer plus hydronephrosis with or without pelvic nodal involvement. J Formos Med Assoc Taiwan Yi Zhi. 2017;116:689–96.
5. Agarwal PK, Kamat AM. Pros and cons of radical cystectomy in the treatment of T1G3 bladder cancer. Indian J Urol IJU J Urol Soc India. 2008;24:77–83.
6. Kay R, Rosten PM, Humphries RK. CD24, a signal transducer modulating B cell activation responses, is a very short peptide with a glycosyl phosphatidylinositol membrane anchor. J Immunol. 1991;147:1412–6.
7. Suzuki T, Kiyokawa N, Taguchi T, Sekino T, Katagiri YU, Fujimoto J. CD24 Induces Apoptosis in Human B Cells Via the Glycolipid-Enriched Membrane Domains/Rafts-Mediated Signaling System. J Immunol. 2001;166:5567–77.

8. Humphrey PA, Moch H, Ulbright TM, Reuter VE. The 2016 WHO classification of tumors of the urinary system and male genital organs- part b.Prostate and bladder tumors. *Eur Urol*. 2016;70(1):106–119
9. Choi YL, Lee SH, Kwon GY, et al. Overexpression of CD24: association with invasiveness in urothelial carcinoma of the bladder. Arch Pathol Lab Med. 2007;131(2):275-81.
10. Kristiansen G, Schluns K,YongweiY, Denkert C, Dietel M, Petersen I. CD24 is an independent prognostic marker of survival in nonsmall cell lung cancer patients. *Br J Cancer.* 2003;88:231–36.
11. Kristiansen G, Denkert C, Schluns K, Dahl E, Pilarsky C, Hauptmann S. CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. *Am J Pathol.* 2002;161:1215–1221.
12. Choi YL, Kim SH, Shin YK, et al. Cytoplasmic CD24 expression in advanced ovarian serous borderline tumors. *Gynecol Oncol.* 2005;97:379–386.
13. Newman H, Shapira S, Spierer O, Kraus S, Rosner M, Pri-Chen S, Loewenstein A, Arber N and Barak A: Involvement of CD24 in angiogenesis in a mouse model of oxygen-induced retinopathy. Curr Eye Res 37: 532-39, 2012.