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Original Research Article

Immunohistochemical Correlation with Morphological Evolution of Urinary Bladder Tumors

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Abstract

Context: Urinary bladder diagnosis can be challenging and conventional markers used were cytokeratins. The new additions are GATA 3, uroplakin and S100P. This study helps to analyze immunohistochemical expression of panel of markers across the spectrum ofurinary bladder tumors.

Aims: 1. to evaluate immunohistochemical expression in various subtypes of urothelial neoplasms of the urinary bladder and to correlate this expression with the grade of the tumor.

2. To distinguish between reactive urothelium and carcinoma in situ/ dysplasia using immunoprofile work up.

Settings and Design: Study was performed in the department of Pathology in RGCIRC, Delhi with 70 cases of urinary bladder lesions during period of one year.

Methods and Material: longitudinal study

Statistical Analysis: The tissue sections were scored using CK, UROPLAKIN II, GATA 3, S100P, CD44, p16 and KI 67

Results: 1. CK7+ and CK20+in 92% and 77% cases. p16+ in 83% and CD 44+ in 84%. Newer markers: Uroplakin II-75%; S100P-82% and GATA3-96%positivity.

CD44+ in reactive urothelium 5/5 and CK20+ in CIS (12/12) cases.

p16+ in 4/5 cases of reactive atypia and CIS 12/12 cases.

Conclusions: The markers are sensitive for urothelial origin & have to be used as panel wherever required.

Keywords: Uroplakin II; Gata 3; Bladder Carcinoma.

Introduction

Urinary bladder cancer is ninth most common cancer in world with approximately 356,000 new cases each year; it is the seventh most common malignancy in men and seventeenth in women. Furthermore, approximately 145,000 patients die from this disease worldwide per year [1]. In India, according to the recentreports of the National Cancer Registry Programme, the overall incidence rate of the urinary bladdercancer is 2.25% (3.67% amongmales and 0.83% for females) [2]. Majority of bladder tumors originatefrom transitional epithelium or urothelium [3].

Approximately 75 85% bladder cancer patients present with disease confined to the mucosa, have a prolonged clinical course in which the patientexperiences multiple

recurrences after local resection without tumor progression. In contrast, a smaller but significant percentage of patients have advanced and muscle infiltrative tumor at the time of diagnosis [4]. The prognosis depends largely on the histological grade and stage of the tumor at diagnosis [5].

The diagnosis of urothelial carcinoma can be challenging in several arenas of both primary andmetastatic setting. Similarly diagnosing dysplasia/ urothelial carcinoma in situ is at times challenging. Theaccurate recognition of carcinoma in situ is important for appropriatepatient management, since those with carcinoma in situ are atincreased risk of invasive urothelial carcinoma. Immunohistochemical markers have shown some utility to resolve these and reach an effective diagnosis. Over the past 2 decades, a variety of immunohistochemical markers, which aid in these diagnostic dilemmas have become available. Conventional markers of urothelial histogenesis are p63 along with coexpression of cytokeratins 7 and 20. The new addition to this list in recent years are GATA 3, uroplakin II and III and S100P [6-8]. This study, has been an endeavour to analyze immunohistochemical expression of panel of markers across the spectrum ofurinary bladder tumors.

Aims and Objectives

1. To evaluate immunohistochemical expression in various subtypes of urothelial neoplasms of the urinary bladder and to correlate this expression with the grade of the tumor.
2. To distinguish between reactive urothelium and carcinoma in situ/ dysplasia using immunoprofile work up.

Material and Methods

It was a longitudinal study based on analysis of histological features of lesions of urinary bladder by two experienced pathologists and to correlate histology with immunoexpression and subsequently establish the utility of a particular marker.

Study was performed in the department of Pathology in Rajiv Gandhi Cancer Institute and Research centre, Delhi after approval from institutional board review and comprises of 70 cases of urinary bladder lesions during period of one year.

Inclusion Criteria

1. Features of reactive epithelium
2. Features dysplasia/carcinoma in situ
3. Features of low grade neoplasm
4. Features of high grade neoplasm

Exclusion Criteria

- Cases with irretrievable data and blocks
- The following panel of markers was used
- CK7, CK20, Ki67, UROPLAKIN II, GATA3, S100P, p16, CD 44.

Antibody	Clone	Dilution	Manufacturer
CK7	OV-TL12/30	1:200	Thermo
CK20	Ks20.8	1:100	DAKO
p16	E6H4™	Ready to use	Ventana
Ki 67	Mib 1	1:100	DAKO
CD44	L178	1:640	Pharmingen
UROPLAKIN II	BC21	Ready to use	Biocare
GATA3	L50-823	1:100	Biocare
S100P	16/F5	Ready to use	Cell Marque

Analysis of Immunohistochemistry

The tissue sections were scored assessing staining intensity localization, including membrane, cytoplasmic, or nuclear localization.

Scoring was performed by two independent observers and the discordant cases were reanalyzed to reach a consensus.

The normal expression patterns, i.e., cytoplasmic staining for cytokeratins , membranous and cytoplasmic for uroplakin II and nuclear for GATA 3, nuclear and cytoplasmic for S100P, membranous staining for CD44, nuclear and cytoplasmic for p 16 were considered for statistical analysis.

In all cases the pattern of immunoreactivity for each antibody determined with regard to basal, intermediate and superficial cell staining of the layers of the neoplastic urothelium.

The expression of these markers evaluated on the following parameters:

1. Percentage of tumor population stained
2. Intensity of staining

Scoring of each parameter done from 0 to 3.

Percentage of tumor population stained

0 for ≤ 10 % of tumor population

1 for 11 to 25% of tumor population

2 for 26 to 50% of tumor population

3 for 51 % and above

Intensity of staining

0 for the null pattern, in which there are absolutely no positive tumour cell nuclei (and a positive internal control is present)

1 for weak

2 for moderate

3 for strong

The reactivity within atypical cell population scored from 0 to 3 for each parameter and then the two scores added to generate a net score .

To quantify Ki67 / proliferation index (PI)

Positive nuclei (weak, moderate, strong) in the hot spots were counted and index calculated bydividing the number of positive cells to total cells in a 40× microscope field .

Observations

70 cases of urothelial lesions within a period of 12 months were studied. Microscopic examination pertaining to morphology along with interpretation of grade of tumor in light of panel of IHC markers was done.

Age Distribution

The 70 cases were divided into 3 groups according to their age.

Table 1 shows that most patients of urothelial carcinomas show mean age of 60 years, However cases diagnosed as PUNLMP show a lower mean age 39.5 years

Table 1: Age Distribution

	Mean	Median
HG	61.2	63.0
LG	60.2	60.0
CIS	59.1	61.5
Punlmp	39.5	39.5
Dysplasia	62.0	63
Benign	57.6	58

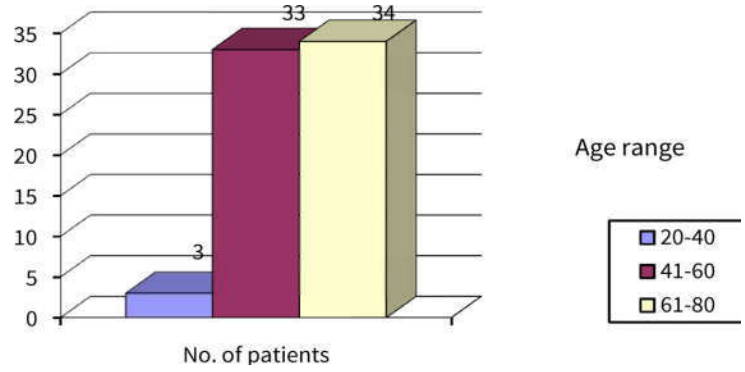

Fig. 1: Majority of the patients were above 40 years of age

Table 2: CK7- Combined Score

		0	≤4	>4	Total	
HG	No. of cases	2	0	23	25	
	% of cases	8%	0.0%	92%	100.0%	
LG	No. of cases	0	0	14	14	
	% of cases	0%	0.0%	100.0%	100.0%	
CIS	No. of cases	0	1	11	12	
	% of cases	0%	8.3%	91.7%	100.0%	
Punlmp	No. of cases	0	0	2	2	*P* value 0.575
	% of cases	0%	0.0%	100.0%	100.0%	
Dysplasia	No. of cases	0	0	3	3	
	% of cases	0%	0.0%	100.0%	100.0%	
Benign Lesion	No. of cases	0	0	14	14	
	% of cases	0%	0.0%	100.0%	100.0%	
Total	No. of cases	2	1	67	70	
	% of cases	2.8%	1.4%	95.7%	100.0%	

CK 7 shows an overall positivity in 97% (68/70) cases in full thickness of urothelium.

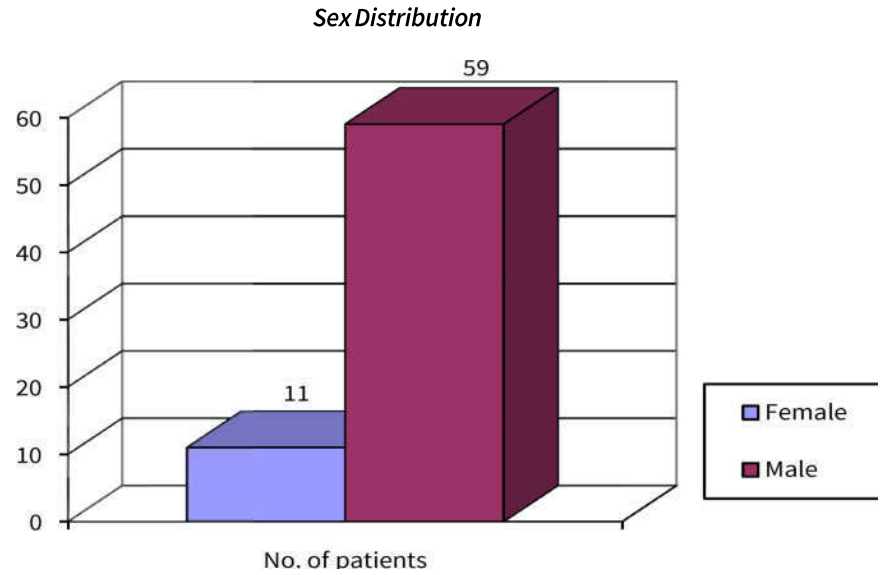

Fig. 2: Distribution of patients according to sex

Table 3: CK20 -Combined Score

		0	1-4	5-6	Total	
HG	No. of cases	4	14	7	25	
	% of cases	16.0%	56.0%	28.0%	100.0%	
LG	No. of cases	4	0	10	14	
	% of cases	28.6%	0.0%	71.4%	100.0%	
CIS	No. of cases	0	0	12	12	
	% of cases	0.0%	0.0%	100.0%	100.0%	*P* value 0.000
Punlmp	No. of cases	0	0	2	2	
	% of cases	0.0%	0.0%	100.0%	100.0%	
Dysplasia	No. of cases	0	0	3	3	
	% of cases	0.0%	0.0%	100.0%	100.0%	
Benign Lesion	No. of cases	8	0	6	14	
	% of cases	57.1%	0.0%	42.9%	100.0%	
Total	No. of cases	16	14	40	70	
	% of cases	22.9%	20.0%	57.1%	100.0%	

CK 20 shows an overall positivity of 77% (54/70)

Table 4: CD44-Combined Score

		0	1-4	5-6	Total	P value
HG	No. of cases	8	1	16	25	
	% of cases	32.0%	4.0%	64.0%	100.0%	
LG	No. of cases	3	0	11	14	
	% of cases	21.4%	0.0%	78.6%	100.0%	
CIS	No. of cases	0	0	12	12	0.109
	% of cases	0.0%	0.0%	100.0%	100.0%	
Punlmp	No. of cases	0	0	2	2	
	% of cases	0.0%	0.0%	100.0%	100.0%	
Dysplasia	No. of cases	0	0	3	3	
	% of cases	0.0%	0.0%	100.0%	100.0%	
Benign Lesion	No. of cases	0	1	13	14	
	% of cases	0.0%	7.1%	92.9%	100.0%	
Total	No. of cases	11	2	57	70	
	% of cases	15.7%	2.9%	81.4%	100.0%	

CD 44 shows an overall positivity of 84% (59/70)

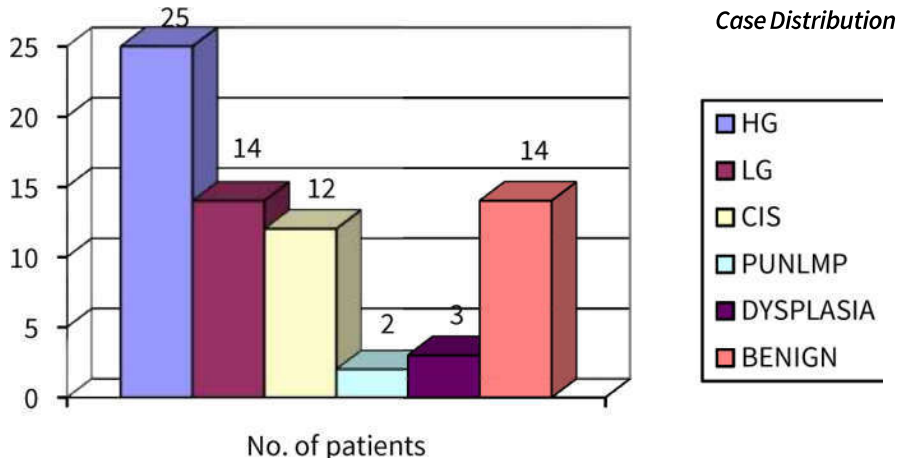

Fig. 3: Case Distribution

Table 5: p 16- Combined Score

		0	1-4	5-6	Total	*P* value
HG	No. of cases	4	0	21	25	
	% of cases	16.0%	0.0%	84.0%	100.0%	
LG	No. of cases	0	1	13	14	
	% of cases	0.0%	7.1%	92.9%	100.0%	
CIS	No. of cases	0	0	12	12	0.000
	% of cases	0.0%	0.0%	100.0%	100.0%	
Punlmp	No. of cases	1	1	0	2	
	% of cases	50.0%	50.0%	0.0%	100.0%	
Dysplasia	No. of cases	2	0	1	3	
	% of cases	66.7%	0.0%	33.3%	100.0%	
Benign Lesion	No. of cases	5	8	1	14	
	% of cases	35.7%	57.1%	7.1%	100.0%	
Total	No. of cases	12	10	48	70	
	% of cases	17.1%	14.2%	68.6%	100.0%	

p 16 shows an overall positivity of 83%(58/70)

Table 6: Ki 67 Index (Proliferation Index ,PI)

	<10%	10-30%	30-50%	>50%	Total
HG	0	0	4	21	25
	0.0%	0.0%	16.0%	84%	100%
LG	6	7	1	0	14
	42.9%	50%	7.1%	0.0%	100%
CIS	9	3	0	0	12
	75%	25%	0.0%	0.0%	100%
Punlmp	0	2	0	0	2
	0.0%	100%	0.0%	0.0%	100%
Benign	14	0	0	0	14
	100%	0.0%	0.0%	0.0%	100%
Total	30	16	3	21	70
	42.9%	22.9%	4.2%	30%	100%

Ki 67 index (proliferation index,PI)- calculated in hot spots

High grade carcinomas PI > 50% in 84%(21/25)

Low grade carcinomas PI < 50% in 58% (8/14)

PUNLMP PI <30% in 100% (2/2)

No significant difference was seen between carcinoma in situ/dysplasia and reactive cases

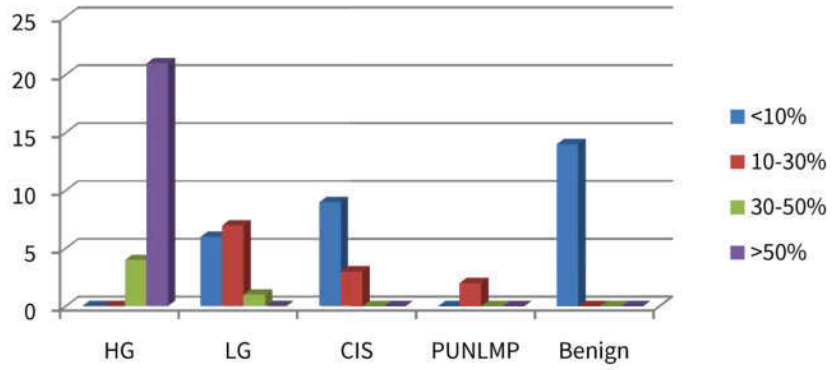

Fig. 4: Ki 67 index according to diagnosis

Table 7: Uroplakin II -Combined Score

		0	1-4	5-6	Total	*P* value
HG	No. of cases	13	1	11	25	
	% of cases	52.0%	4.0%	44.0%	100.0%	
LG	No. of cases	0	0	14	14	
	% of cases	0.0%	0.0%	100.0%	100.0%	
CIS	No. of cases	0	2	10	12	
	% of cases	0.0%	16.7%	83.3%	100.0%	
Punlmp	No. of cases	0	0	2	2	0.001
	% of cases	0.0%	0.0%	100.0%	100.0%	
Dysplasia	No. of cases	1	0	2	3	
	% of cases	33.3%	0.0%	66.7%	100.0%	
Benign Lesion	No. of cases	4	0	10	14	
	% of cases	28.6%	0.0%	71.4%	100.0%	
Total	No. of cases	18	3	49	70	
	% of cases	25.7%	4.3%	70.0%	100.0%	

Uroplakin II shows an overall positivity of 75% (52/70) limited to umbrella cell layer.

Table 8: GATA3- Combined Score

		0	5-6	Total
HG	No. of cases	3	22	25
	% of cases	12%	88%	100.0%
LG	No. of cases	0	14	14
	% of cases	0.0%	100.0%	100.0%
CIS	No. of cases	0	12	12
	% of cases	0.0%	100.0%	100.0%
Punlmp	No. of cases	0	2	2
	% of cases	0.0%	100.0%	100.0%
Dysplasia	No. of cases	0	3	3
	% of cases	0.0%	100.0%	100.0%
Benign Lesion	No. of cases	0	14	14
	% of cases	0.0%	100.0%	100.0%
Total	No. of cases	3	67	70
	% of cases	4.2%	95.7%	100.0%

GATA 3 shows an overall positivity of 96% (67/70)

Table 9: S100P- Combined Score

		0	1-4	5-6	Total	*P* value
HG	No. of cases	10	5	10	25	
	% of cases	40.0%	20.0%	40.0%	100.0%	
LG	No. of cases	0	1	13	14	
	% of cases	0.0%	7.1%	92.9%	100.0%	

CIS	No. of cases	1	1	10	12	0.024
	% of cases	8.3%	8.3%	83.3%	100.0%	
Punlmp	No. of cases	0	0	2	2	
	% of cases	0.0%	0.0%	100.0%	100.0%	
Dysplasia	No. of cases	1	0	2	3	
	% of cases	33.3%	0.0%	100.0%	100.0%	
Benign Lesion	No. of cases	1	4	9	14	
	% of cases	7.1%	28.6%	64.3%	100.0%	
Total	No. of cases	13	11	46	70	
	% of cases	18.6%	15.7%	65.7%	100.0%	

S100P shows an overall positivity of 82% (57/70)

Discussion

In this study 70 cases of urothelial lesions were analyzed histomorphologically. Panel of I.H.C. markers were applied and different parameters pertaining to the stain were assessed. Majority of lesions studied occurred in males 84.3% (59/70) and most of the malignant tumors also occurred in males 89.7% (35/39). This finding was comparable to different studies which also showed a male predominance. In the study by Paliwal et. al. [3] there was a male predominance with 79% cases being male and the rest 21% females.

The overwhelming male predominance in this study can be attributed to smoking and occupational exposure of carcinogens in Indian males, which are risk factors for urothelial cancer [9-11].

The age range in our study showed a wide spectrum from 21-80 years with a median age of 63 in high grade tumors, 60 in low grade tumors, and 39.5 in PUNLMP. These findings were comparable to other studies. The age of patients ranged between 32 and 80 years (median= 62 years) in the study by Paliwal et. al. [3] which was very similar to our figures. They reported that incidence of transitional cell bladder carcinoma had an upward trend, most cases were diagnosed in the 6th and 7th decades of age.

In our study 35.7% (25/70) were diagnosed as high grade urothelial carcinomas which included infiltrating urothelial carcinoma and one case each of variants (nested, sarcomatoid, squamous). Followed by 19.9% (14/20 of low grade urothelial tumors, 17.1% (12/70) carcinoma in situ, 4.3% (3/70) dysplasia and 2.9% (2/2) PUNLMP. Of the 20.1% (14/70) benign lesions; reactive atypia was 7.1% (5/14), hyperplasia was 13% (9/14)

This study evaluated these lesions for traditional markers of urothelial histogenesis viz CK7, CK20 and novel markers viz CD44, p16, Uroplakin II, GATA3 and S100P Panel also included Ki 67 as marker for proliferation.

Traditional Markers

CK7

CK7 expression was seen in 92% (68/70) cases. Cytoplasmic positivity in full thickness of urothelium was seen. Of high grade carcinoma 92% (23/25) cases showed positivity.

This is in concordance with most other studies by different scholars like Jiang et. al. who reported CK7 immunoreactivity in all primary urothelial cancers [12]. Mhawech et. al. [13] found CK7 expression in 86.6% of UC and Chu et. al. [14] described 88% positivity in urothelial carcinoma.

Loss of expression was seen in sarcomatoid urothelial carcinoma and neuroendocrine carcinoma. Nested variant and squamous differentiation showed positivity. The only study describing the positivity in variants by Paner et al showed reduced expression in variants of urothelial carcinoma upto 30% in variants [15].

CK20

In our study 77% (54/70) cases were positive and in HG carcinomas 84% (21/25) cases showed positivity of mild intensity.

In low grade carcinomas 71.4% (10/14) cases showed positivity of variable patterns with 50% (7/14) cases in curtain down pattern (superficial to basal layer).

Decrease in intensity with increasing tumor grade was noted.

Likewise, in the study by Jiang et. al. [12], CK20 expression was seen in 46% primary tumor and Mhawech et. al. [13] demonstrated positivity in 66.6% of urothelial carcinoma cases. In our study sarcomatoid urothelial carcinoma showed loss of expression while neuroendocrine, nested variant showed mild positivity. Similarly the only study by Paner et al [15] cites 6% positivity in these variants.

CK 20 has been used by many pathologists in along with other markers for distinguishing reactive urothelium from dysplasia/CIS cases. In our study when reactive cases were assessed, 40% (2/5) cases showed focal positivity in umbrella cell layer. In contrast, 100% Carcinoma in situ and 100% (3/3) dysplasia exhibited positive staining in full thickness of urothelium. Fifty percent cases of CIS cases showed > 50% of cells stained with 2+/3+intensity.

Similar findings were reported by Mckenney et. al. [16] who used a panel of CK20, CD44, p53. They cited CK 20 immunoreactivity in only umbrella cell layer in all (15/15) cases of reactive urothelium and in contrast intense positivity in 81% of carcinoma in situ cases in majority (>50%) of malignant cells [16].

CD44

CD 44 is another marker proposed by many scholars to be used in panel when CIS and reactive cases are to be segregated. In the present study it was expressed in entire reactive urothelium in 80% cases (4/5). In contrast carcinoma in situ cases, showed basal cell layer staining in 42% (5/12) cases, and the remaining 58% (7/12)cases showed CD44 immunoreactivity in basal cell layer along with intermediate cell layers. Dysplasitic urothelium showed basal layer staining in 100%(3/3) cases Similarly McKennney et al reported CD44 overexpression in all the layers in reactive urothelium in 100% (15/15) cases and basal layer staining in 44% of carcinoma in situ cases [16].

In addition to this in our study CD 44 immuno expression was seen in 52% of high grade tumors, 78.6% of low grade tumors and100% of PUNLMP. In all these lesions moderate degree of intensity was observed in basal as well as intermediate layers. Increase in positivity (percentage of cells) with decreasing tumor grade was observed. Our results were comparable with the study by Desai et. al. [17]. According to their study 50%, 38.1% and 12.1% positivity in PUNLMP, low grade and high grade papillary neoplasms was seen respectively. They also observed immunoexpression in the suprabasal intermediate cells, as well as in the basal cells, but not in the superficial cells.

According to present study CD 44 along with other markers like CK 20 and p16 can be of aid in distinguishing reactive atypia from carcinoma in situ. Immunoexpression of CD 44 is inversely related to the WHO/ISUP grade in majority of cases.

p 16

p 16 expression was seen in 84% cases. Nuclear with cytoplasmic reactivity was observed. High grade carcinomas showed positivity in 88% (21/25) cases with 76% (19/25) cases showing strong intensity. Variants (nested, sarcomatoid, squamous) and neuroendocrine carcinoma showed loss of expression.

Low grade carcinomas showed 100% (14/14) positivity with mild to moderate intensity in 58% (8/14) cases and PUNLMP showed positivity of mild intensity in 50% (1/2) cases. Increase in positivity (% of cells as well as intensity) was seen with increasing tumor grade.

Study by Nakazawa et. al. [18] also showed that in low-grade urothelial carcinoma, 32% cases showed positivity (moderate intensity) and in high grade noninfiltrating urothelial carcinoma, 58% cases overexpressed p 16.

Carcinoma in situ cases show positivity in 100% cases with 58% showing 3 + intensity and dysplastic urothelium showed only 34% (1/3) case positivity of 2+ intensity.

In contrast only 50% of the benign lesions showed focal positivity and 38% showed null expression. Likewise in study by Nakazawa et. al. [18] 70% carcinoma in situ cases showed overexpression and among nonneoplastic lesions, 44% showed focal immunoreactivity and 49% cases were negative for p16. So present study suggests role of p 16 immunoexpression in grading urothelial carcinoma and in differentiating reactive atypia from carcinoma in situ

Ki 67

Ki 67 index was calculated in the hot spots. Ki 67 index ranged from 40% to 90% in high grade carcinomas. 10% to 30% in low grade carcinomas and PUNLMP. Similar to this Helpap et. al. [19] cited that Ki67 proliferation index is increased in high-grade carcinoma with or without invasion. However no standard cut off guidelines have been established

In present study no significant difference was seen between carcinoma in situ and reactive cases however studies by Gunia S et . al. [20] have concluded that 16% or more Ki67+ve nuclei favoured CIS and less than 15% favoured dysplasia or reactive atypia.

Our study does'nt suggest Ki 67 index to be useful in grading of urothelial tumors as the range of high grade and low grade is quite wide.

Uroplakin II

Present study reveals Uroplakin II expression in 75% (52/70) urothelial cases.

Uroplakin II antibody was used in our study as previous studies by Hoang et. al. [21] and Smith et. al. [22] have said that UPII has greater sensitivity than UPIII and the cytoplasmic and membranous staining of UPII is more diffuse than plaque like staining of UPIII.

Uroplakin II was found to be positive in 66.67% (26/39) of malignant (including both high grade and low grade) cases. Authors like Smith et. al. [22] and Hoang L et . al. [21] reported 68% and78% positivity in urothelial cancers respectively.

Among the high grade carcinoma only 48% (12/25) cases showed positivity and variants (sarcomatoid, squamous) and Neuroendocrine carcinoma showed loss of expression while low grade carcinomas (14/14) and PUNLMP were 100% (2/2) positive .Decrease in positivity was seen with increasing tumor grade. Likewise, in study by Hoang et. al. [21] uroplakin II was expressed in 88%, 82%, 55% of grade 1, 2, 3 urothelial carcinomas, respectively. Regarding variants Paner et. al. [15] cited null expression. Previous studies [21-24] have shown that

the percentage of positivity has ranged from 14% to 78% for uroplakin III which indicates poor sensitivity. Also to note here is that loss of expression in high grade tumors qualifies uroplakin to be used in a panel rather than alone.

GATA 3

GATA 3 showed an overall positivity of 96% (67/70) cases.

88% (22/25) cases of high grade carcinomas showed nuclear positivity in all the layers of urothelium. This was in concordance with the results of studies by scholars like Chang A et . al.[25] who reported positivity in invasive high grade urothelial carcinomain 80% cases and Higgins et. al. [26] reported strong positive rate in 81.6% urothelial carcinomasVariants of high grade carcinoma (sarcomatoid and urothelial carcinoma with squamous differentiation) and neuroendocrine carcinoma showed loss of expression while nested variant showed positivity. However Paner et . al. [15] reported 20% of expression in variants of high grade carcinoma. GATA3 as per the present study has shown good positivity and proves to be sensitive marker of urothelial origin but as cited by many authors to be less specific for urothelium [25,26] can be used along with other markers in particular scenarios like differentiating from high grade prostatic carcinoma from high grade urothelial carcinoma.

S100P

In our study we found that, S100P was expressed in 82% (57/70) cases of urothelial lesions. In high grade carcinomas 60% (15/25)cases showed positivity while low grade tumors and PUNLMP showed 100%(16/16) positivity.

Decrease in positivity (% of cells and intensity) with increasing tumor grade was seen. Similar results were observed by Higgins et al with S100P immunoexpression in 78% of urothelial carcinoma and decreased staining with increasing tumor grade with positivity in 71.2% of high grade tumors and 96.3% low grade tumors [26].

Variants (squamous, sarcomatoid) and neuroendocrine carcinoma showed focal positivity of mild intensity. Similarly, Paner et al reported positivity of mild intensity in the variants of high grade carcinoma [15].

The results of S100P in the present reveal that S100P has variation of expression across the WHO grades, so it might be helpful in that respect; but due to variation of positivity as quoted in previous studies this marker is not specific for urothelium. It should be used in the panel depending upon the relevant context.

Summary

Evaluation of Markers

1. CK7 and CK20 considered the traditional markers showed 92% and 77% positivity respectively.
2. p 16 showed 83% positivity and CD 44 showed 84% positivity.
3. Newer markers: Uroplakin II showed 75% (52/70) positivity; S100P showed 82% (57/70) positivity and GATA3 showed 96% (67/70) positivity.

Markers for Differentiating Reactive Atypia from Carcinoma in Situ

1. CD44 showed overexpression in reactive urothelium in 100% (5/5) cases. In contrast in carcinoma in situ the expression was confined to basal cell layer in 58% (7/12) cases.
2. CK20 showed focal positivity in reactive urothelium in 40% (2/5)cases restricted to umbrella layer and remaining 60%(3/5) were negative. In contrast in carcinoma in situ full thickness expression in 100% (12/12) cases was seen.
3. P 16 showed focal positivity of mild intensity in 80% (4/5) cases of reactive atypia while in carcinoma in situ positivity of moderate to severe intensity in 100% (12/12) cases was observed.

Conclusion

The observations and results indicate that above markers are sensitive for urothelial origin and they have to be used in conjunction with each other. CK 20, CD 44, p16 can supplement the decision making in differentiating reactive atypia from dysplasia/carcinoma in situ

Key Message

IHC is useful in establishing origin, grading the neoplasm as well as diagnosing dysplasia whenever histopathologist is in dilemma regarding Hand E stained morphological features.

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