

ROLE OF CK20, CK5/6 and P53 in the Diagnosis of Flat Urothelial Lesions with AtypiaHayam E. Rashed¹, Eman H. Abdelbary¹, Ahmed Ragab Ali.²Pathology¹ and Urology² Departments, Faculty of Medicine, Zagazig University, Egypt
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Abstract: Background: Diagnosis of carcinoma *in situ* in bladder specimens is of great benefit because it has prognostic and therapeutic value. Morphology alone may not be sufficient in the differentiation of reactive urothelial atypia (RUA), urothelial dysplasia (UD) and carcinoma *in situ* (CIS). Specific markers to enhance morphology would be of great value in differentiation of RUA from CIS and UD. **Objectives:** We aim to determine the utility of a selected panel of markers (CK20, CK5/6 and P53) as an adjunct in the diagnosis of reactive urothelial atypia, urothelial dysplasia and carcinoma *in situ* by comparing their results with the histopathological finding in the follow up cystoscopic biopsy; and help to reach a definite diagnosis in atypia of unknown significance (AUS). **Methods:** A case-controlled study included 60 patients were selected from Urology outpatient clinic, Faculty of Medicine, Zagazig University, Egypt. Fifty cystoscopic biopsy specimens of flat urothelial lesions and 10 of normal urothelium as (control) were examined immunohistochemically using antibodies against Cytokeratin20, CK5/6 and P53. They were also enrolled in the follow up schedule which was planned according to histopathological finding. **Results:** All normal urothelia showed normal staining patterns with CK20, CK5/6 and P53. In the CIS group, 84.6%, 100% and 69.2% of cases showed abnormal expression pattern with CK20, CK5/6 and P53 respectively. Regarding dysplasia group, 81.8%, 100% and 54.5% of cases showed abnormal expression with CK20, CK5/6 and P53 respectively. In the AUS group, 50% showed abnormal CK20, increased P53 expression and negative CK5/6, all were suggestive of urothelial dysplasia; the remaining cases were thought to be (RUA). The follow up results were comparable with the immunohistochemical finding. **Conclusions;** CK20, CK5/6 and P53 are promising to be reliable diagnostic markers of UD and CIS in conjunction with morphological changes especially in cases of diagnostically challenging biopsies and help to reach a definite diagnosis in AUS cases. Ck20 only cannot differentiate between UD and CIS, in biopsies of flat intraurothelial lesions with atypia.

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Key Words: Dysplasia, Carcinoma in situ, Reactive atypia, Atypia of unknown significance, Cytokeratin 20, CK5/6, P53, Immunohistochemistry.

Abbreviations: CIS: Carcinoma in situ; RUA: Reactive urothelial atypia; AUS: Atypia of unknown significance; UD: Urothelial dysplasia.

1. Introduction

Bladder cancer is a worldwide public health problem. It ranks 4th and 10th among the commonest cancers in men and women respectively.¹ Egypt has the highest incidence of bladder cancer in the world; Egyptian males' bladder cancer mortality rates was (16.3 per 100,000) which exceed that in the United States (3.7) and Europe (8.0 in Poland and 8.3 in Spain).² The typical cost per patient with bladder cancer from diagnosis to death was estimated to be the highest among all cancers. Early diagnosis of bladder lesions may decrease the costs and ultimately leads to decreased bladder cancer mortality and morbidity.³

Flat urothelial lesions with atypia were classified as reactive urothelial atypia, urothelial dysplasia, urothelial atypia of unknown significance, and urothelial carcinoma in situ by the 2004 World Health Organization (WHO) consensus committee.⁴ Flat lesions like (CIS), may be missed during cystoscopy so to improve its detection, Photodynamic diagnosis is performed, using blue light after Vesical

instillation with hexaminolaevulinic acid (Hexvix[®]) to ameliorate urothelial lesions.⁵ Multiple cup biopsies from bladder are indicated for; (a) previously diagnosed superficial bladder under the follow up schedule (b) papillary bladder cancer for detection of flat multifocality lesions, (c) Patients presented with dysuria, hematuria, increased frequency, positive urine cytology or at high risk of development of bladder cancer, to detect (CIS). (d) Patients presented with persistence urinary symptoms (dysuria, frequency, and hematuria) but did not respond to routine medical treatment.⁶ The follow up cystoscopy must be continued after treatment 5 year bladder tumor-free.⁷

Reactive urothelial atypia is a benign condition characterized by minimal nuclear changes occurring in chronically or acutely inflamed urothelium. Most patients with reactive atypia have a history of cystitis, infection, stones, instrumentation, or prior treatment and present with hematuria and/or irritative symptoms. Under cystoscopic evaluation, the urothelium look erythematous or inflamed.

Microscopically, the urothelium may be normal or slightly thickened, but it keeps the maturation pattern from superficial to basal cells. Cells are usually bigger than normal, with exuberant cytoplasm and uniformly enlarged, vesicular nuclei that may have prominent nucleoli.^{8,9}

“Atypia of unknown significance” is a term used when cytologic and architectural changes in the urothelium are less than that of dysplasia. It describes lesions with nuclear abnormalities similar to those of reactive changes, but not in harmony with the degree of the causative agent. Patients with AUS mostly present with irritative symptoms or hematuria. In contrast to reactive atypia, they usually have a past history of urothelial dysplasia or subjected to intravesical treatment, such as immunotherapy, chemotherapy or radiotherapy.^{10,11}

Urothelial dysplasia is defined as the loss of polarity with nuclear rounding and crowding and cytologic atypia that is not severe enough to diagnose CIS. CIS and UD are precursor lesions of invasive urothelium carcinoma and their detection, especially CIS, is associated with a significant risk of progression and recurrence.^{8,12}

CIS is often multifocal and can occur in the upper urinary tracts and in the prostatic ducts and urethra. CIS exists in two settings; isolated (primary) CIS and secondary CIS associated with papillary urothelial carcinoma. Isolated CIS was rare, accounting for about 10% of all CIS and 1% to 3% of bladder neoplasm.¹³

Although nuclear and architectural features are the primary criteria for differentiation between dysplasia, CIS and reactive atypia, may be difficult in patients previously treated for CIS. Expression of markers as CK20, CD44, p53, and Ki67 may be helpful.^{14,15}

Cytokeratin 20 is 46 KDa intermediate filament proteins that expressed mainly in gastric and intestinal epithelium, urothelium, and Merkel cells but showing a limited pattern of expression in their normal tissues. So, it may be an important tool for detecting cancer and metastases of these tissues, either by immunohistochemistry or reverse transcription–polymerase chain reaction analysis. Umbrella cell layer typically exhibit CK20 staining identical to normal urothelium.^{14,15} Cytokeratin (CK) 5/6 is present in normal keratinizing epidermis and squamous mucosal epithelium, as well as in basal cells or myoepithelial cells of the breast, salivary glands, and prostate.¹⁶

Differentiating RUA from UD and CIS in the inflamed urothelium is important because of different therapeutic modalities especially in post-therapy changes. Other reasons such as small specimen size and interobserver variability may also

contribute to difficulties in reaching the correct diagnosis.¹⁷ We aim to determine the role of a selected panel of markers; CK20, CK5/6 and P53 as an adjunct in the diagnosis of reactive urothelial atypia, urothelial dysplasia (UD) and carcinoma in situ (CIS) and to resolve cases of (AUS) to reach the accurate diagnosis.

2. Patients and Methods

This case-control study was performed after approval by the local ethical Committee, and a written informed consent was obtained by each patient.

This study included 60 patients were selected from the outpatient clinic of the Urology department and diagnosed at Pathology department Faculty of Medicine, Zagazig University, Egypt, in the period from June 2011 to June 2013. The control group included 10 patients who underwent cold cup biopsies from the urinary bladder while undergoing ureteric stent (before shockwave lithotripsy). Fifty patients were selected according to the clinical presentation as in Table (1). Full clinical examination, routine laboratory investigations, urine Cytology, imaging in the form of abdominal and pelvic ultrasonography, and computed tomography scan with contrast (CT) of the abdomen and Pelvis in selected cases. All patients underwent urethrocytoscopy and biopsies by resectoscope or cold cup biopsies if needed under spinal anesthesia. We examined all urethral, bladder mucosa and both ureteric orifices by cystoscope. We resected any flat lesion then biopsies were taken from the abnormal urothelium. Patients without bladder lesion and had positive cytology or under high risk (heavy smoker, positive family history for bladder cancer and occupational hazards) were subjected to random cold-cup biopsies that were taken from; bladder dome, trigone, right, left, posterior and anterior bladder walls. The biopsy specimens were fixed immediately with formalin 10% and staining with haematoxylin and eosin then were assessed histopathologically and formalin-fixed, paraffin-embedded blocks were done. They were classified according to 2004 World health organization/ pathology /1998 international society of urological classification of urothelial neoplasm.⁴ Clinical presentations of patients are shown in table (1).

Immunohistochemical staining:

Immunohistochemical staining was done by using streptoavidin-biotin immunoperoxidase technique. 3–5 μ m thick sections cut from formalin-fixed, paraffin-embedded blocks of all cases, mounted on positively charged slides, then deparaffinized in xylene and rehydrated in graded alcohol. Sections were boiled in citrate buffer (pH

6.0) for 20 min and then washed in phosphate buffer saline (pH 7.3). Thereafter, blocking of endogenous peroxidase activity with 6% H₂O₂ in methanol was carried out. The slides were then incubated overnight with monoclonal antibodies; CK 20 mouse monoclonal antibody Cat from Thermo Scientific/Lab Vision Corporation, Fermont, USA, clone: Ks20.8. Dilution 1:50), anti-p53, mouse monoclonal antibody (Ab-6, clone DO-1 dilution 1:30 Thermo Scientific Lab Vision). CK 5/6 clone D5/16 B4 Thermo Scientific Lab Vision

Incubation with a secondary antibody and product visualization were performed (Lab Vision Corporation, Fermont, USA), with diaminobenzidine substrate as chromogen. The slides were finally counterstained with Mayer's haematoxylin. Colonic carcinoma was used as a positive control for Ck20 and p53 while mesothelioma was used for Ck5/6. Negative controls, obtained by substitution of primary antibodies with blocking buffer, were included in the staining procedure.

Evaluation of the results of immunohistochemical staining:

The pattern of immunoreactivity for CK20, P53 and CK5/6 was defined as negative or positive as follows;

CK20 positive: in atypical cells shows moderate to strong staining (often in all thickness). CK20 negative: no staining or weak/patchy (usually in umbrella cells).

P53 positive: strong staining to moderate staining (mostly in full thickness) in atypical cells. P53 negative: weak/patchy to no staining (mostly in the intermediate parabasal and basal cells).

CK5/6 positive: moderate to strong staining (mainly in full thickness). CK5/6 negative: staining only the basal/parabasal cell or no staining.¹⁸

Follow up plan was done at 3, 9, 24 month. At the end of the study patients were instructed to continue the follow up in the outpatient clinic yearly for 5 years if the results were negative for dysplasia. But if it were positive the follow up plan was every 3 months till the end of the study, and then continue every 6 months until 5 years and then yearly. Follow up plan included history taking, physical examination, urine cytology, abdominopelvic ultrasound, abdominopelvic computerized tomography with contrast if indicated, cystoscopy and resectoscope if needed.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS, Chicago, version 20 IL, USA). Data were expressed as mean \pm SD for quantitative variables. Fisher's exact test or chi-square was used to analyze the distribution of markers among different

groups. *P*-value less than 0.05 were considered significant.

3. Results

Patient's ages ranged from 44 - 77 years, with a mean of 63.96 ± 7.13 . The majority of studied cases were males (88%). Cytological findings were positive in 22 cases (44%). The major presentation (46%) of the patients was; previously diagnosed papillary carcinoma. Others clinical presentation of patients in relation to histopathological diagnosis was summarized in table (1). Abnormal cystoscopic examination were; done and random biopsies were taken from abnormal and normal urothelium, and treat any associated finding. Dysplasia and CIS were significantly associated with older age (>70years, *p* value<0.005).

Immunoreactivity patterns in normal urothelium.

All cases (100%) of normal urothelia had normal expression patterns with all three antibodies. In normal urothelium, CK20 demonstrated patchy cytoplasmic staining of only the superficial cell layer. P53 nuclear immunoreactivity mostly basal varied from focal to very weak and none, CK5/6 stains only basal cell of normal urothelium.

Immunoreactivity patterns in reactive urothelium.

Reactive atypia showed CK20 staining in only the umbrella cell layer in 95% of cases. The only case that positive for CK20, showed full thickness CK5/6 immunoreactivity and focal P53. Immunostaining for CK5/6 showed diffuse and strong reactivity in 95% of RA cases. P53 stain in all cases of reactive atypia varied from absent to patchy and weak nuclear reactivity, predominantly in the basal cell layer. Tables [2-4] and [Fig.1].

Immunoreactivity patterns in dysplasia.

Regarding dysplasia group, showed 100%, 81.8%, 54.5% abnormal expression pattern with CK5/6, CK20 and P53 respectively. Two cases showed mild CK20 but positive P53 and negative CK5/6. Cases negative for P53 were CK20 positive and CK5/6 negative. Tables [2-4] and [Fig.2].

Immunoreactivity patterns in CIS.

In CIS group, 84.6% (11/13) had full thickness CK20 expression, while two cases showed CK20 positivity only in the upper third of the urothelium accompanied by positive P53 expression and negative for CK5/6. Positive P53 expression was observed in 69.2% (9/13) of cases. The 4cases with negative P53 had Positive CK 20 and negative CK5/6 as shown in table [2-4] and [Fig.3].

Immunoreactivity patterns in AUS.

A total of 3/6 cases (50%) of AUS were CK20 and P53 positive but CK5/6 negative. All these 3 cases were favored to be UD. One case showed moderate P53, focal CK20 and negative CK5/6

expression but this was morphologically benign. The remaining cases were negative for both (CK20 and P53) antibodies but positive for CK5/6 those were thought to be reactive table [2- 4] and [Fig.4A, B].

The follow up schedule; which was started for all cases after 3 months from the original biopsy. Then at 9 month then at the end of the study for about 26% of cases and for the other 74% every 3 months till the end of the study according to, histopathological finding, presence or absence of dysplastic urothelium. At the end of the follow up (after the first and second follow up biopsy), the fate of different flat lesions previously diagnosed by histopathology was; The 6 cases of AUS was categorized as follow (3 cases RUA, 2 cases CIS and

1 case as UD). These follow up findings was similar to immunohistochemical results. CIS detected in 15cases (13originally histopathological diagnosed and 2cases of AUS immunohistochemically diagnosed as CIS) which was confirmed by definitive histopathological diagnosis after the second follow up biopsy. UD in 12 cases (11 cases originally histopathologically diagnosis and one case of AUS immunohistochemically diagnosed as UD) which was confirmed by definitive histopathological diagnosis after the first follow up biopsy. All cases of CIS, UD, RUA with past history of TCC (8 cases) and patients with high risk of bladder cancer continued the follow up regimen after the end of the study.

Table (1): Clinical presentation of patients in relation to histopathological diagnosis.

Patient's presentation	No	Reactive urothelial atypia		Atypia of unknown significance		Dysplasia		Carcinoma in situ	
		No	%	No	%	No	%	No	%
		Patients with previously diagnosed papillary carcinoma (under the follow up schedule)	23	8	34.8%	3	13%	4	17.4%
Patients with hematuria and positive urine cytology	10	0	0.0	2	20%	4	40%	4	40%
Patients with hematuria at high risk	5	1	20%	1	20%	2	40%	1	20%
patients present with unresolved urinary symptoms (hematuria, dysuria, and frequency)	12	11	91.7%	0	0.0	1	8.3%	0	0.0
Total	50	20	40%	6	12%	11	22%	13	26%

Table (2): Immunohistochemical expression of CK5/6, CK20 and P53 in flat urothelial lesions.

Flat Urothelial lesions	No	Ck5/6				CK 20				P53			
		Negative		Positive		Negative		Positive		Negative		Positive	
		No	%	No	%	No	%	No	%	No	%	No	%
Reactive urothelial atypia	20	1	5.0	19	95.0*	19	95.0*	1	5.0	20	100.*	0	0.0
Atypia of unknown significance	6	4	66.7	2	33.3	3	50.0	3	50.0	2	33.3	4	66.7*
Dysplasia	11	11	100.0*	0	0.0	2	18.2	9	81.8*	5	45.5	6	54.5
Carcinoma in situ (CIS)	13	13	100.0*	0	0.0	2	15.4	11	84.6*	4	30.8	9	69.2*
Total	50	29	58.0	21	42.0	26	52.0	24	48.0	31	62.0	19	38.0
X ²		40.63				26.85				21.01			
P		<0.001*				<0.001*				<0.001*			

* Significant P value

Table (3): Ck5/6 and CK20 immune-profile of flat epithelial lesions of the urinary bladder

	No	Ck5/6 and CK20 Immunoprofile of flat lesions								X ²	P
		Ck5/6-ve/CK20-ve		Ck5/6+ve/CK20+ve		Ck5/6 +ve/CK20-ve		Ck5/6-ve/CK20+ve			
		No	%	No	%	No	%	No	%		
Reactive urothelial atypia	20	0	0.0	1	5.0	19	95.00*	0	0.0	42.46	<0.001*
Atypia of unknown significance	6	1	16.7	0	0.0	2	33.3	3	50.0	0.59	0.89
Dysplasia	11	2	18.2	0	0.0	0	0.0	9	81.8*	11.08	0.011*
Carcinoma in situ (CIS)	13	2	15.4	0	0.0	0	0.0	11	84.6*	13.93	0.0029*
Total	50	5	10.0	1	2.0	21	42.0	23	46.0		

* Significant P value

Table (4): CK20 and P53 immune-profile of flat epithelial lesions of the urinary bladder

	No	Immunoprofile of flat lesions								X ²	P
		CK20-/P53-		CK20+/ P53+		CK20+/ P53 -		CK20-/ P53+			
		No	%	No	%	No	%	No	%		
Reactive urothelial atypia	20	19	95.0	0	0.0	1	5.0	0	0.0	38.7	<0.001*
Atypia of unknown significance	6	2	33.3	3	50.0	0	0.0	1	16.7	2.97	0.39
Dysplasia	11	0	0.0	4	36.4	5	45.4	2	18.2	11.79	0.008*
Carcinoma in situ(CIS)	13	0	0.0	7	53.8	4	30.8	2	15.4	13.1	0.004*
Total	50	21	42.0	14	28.0	10	20.0	5	10.0		

* Significant P value

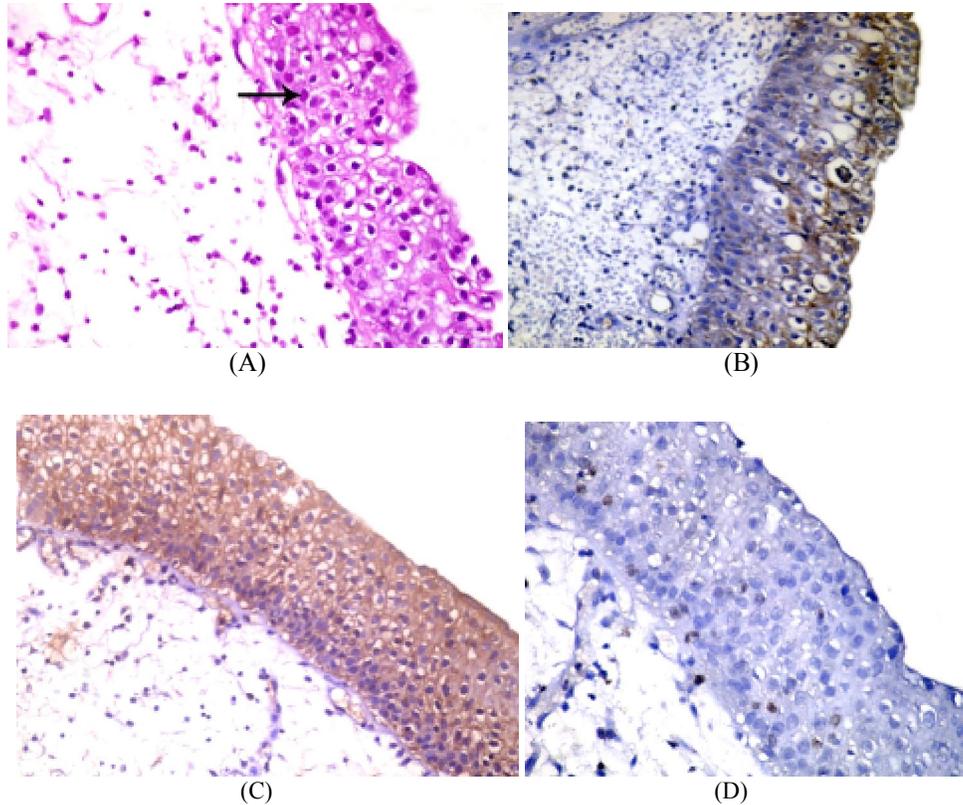
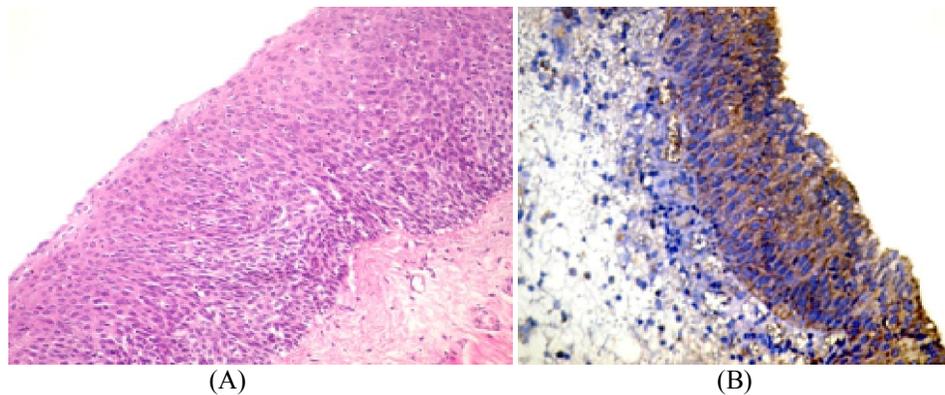


Fig. (1): Reactive urothelium: **(A)** H&E section, showing nuclear hyperchromasia (arrow), but they are relatively uniform in size and show maturation; X 400 **(B)** lack of CK20 staining in reactive cells only in umbrella cells; X400 **(C)** full thickness CK5/6 reactivity X400. **(D)** Weak, patchy mainly basal P53 nuclear staining; X400



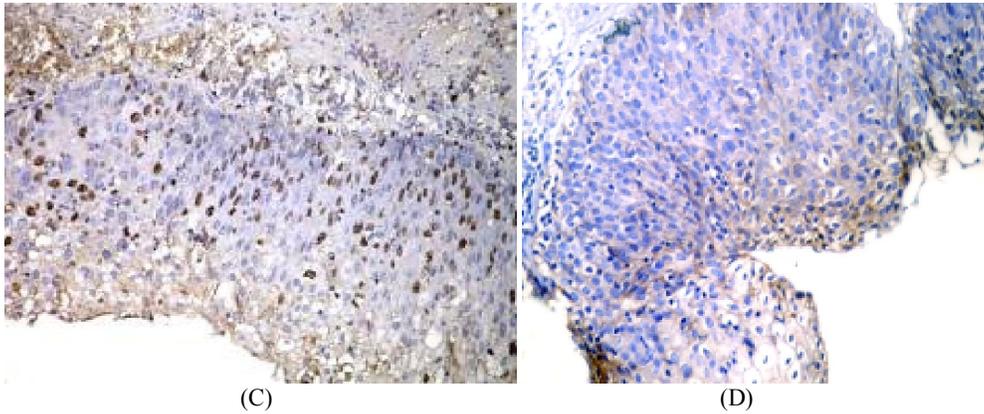


Fig. (2): Urothelial dysplasia. **(A)** H&E section, the thickened urothelium is populated by dysplastic cells that are variable in size and shape, some cellular polarity and maturation present X200. **(B)** Urothelial dysplasia with aberrant cytokeratin 20 expression X400 **(C)** P53 stain highlights dysplastic cells X 200**(D)** Lack of CK5/6 staining in dysplastic cells X 200.

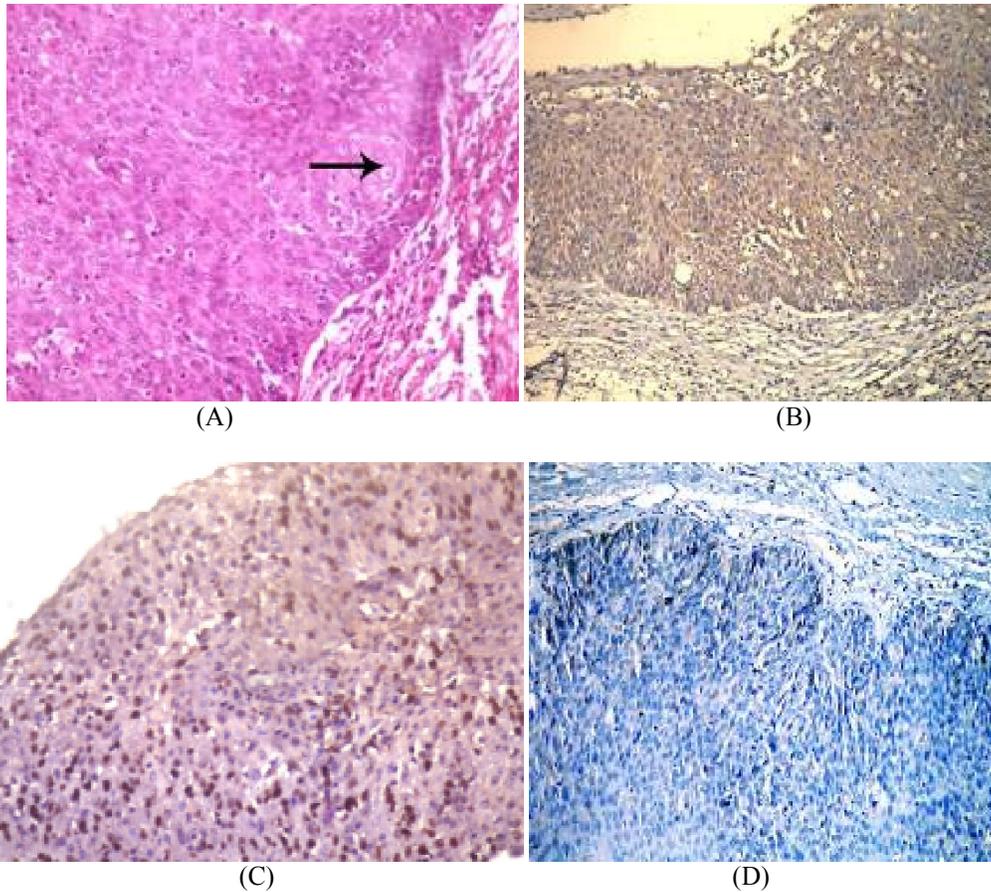


Fig. (3): CIS **(A)** H&E section, showing full-thickness involvement of the urothelium by the carcinoma in situ; X 400 **(B)** CK20 staining the entire thickness of the neoplastic urothelium; X 200 **(C)** diffuse, strong P53 nuclear staining X 400 ; **(D)** lack of CK5/6 staining in neoplastic cell X 200.

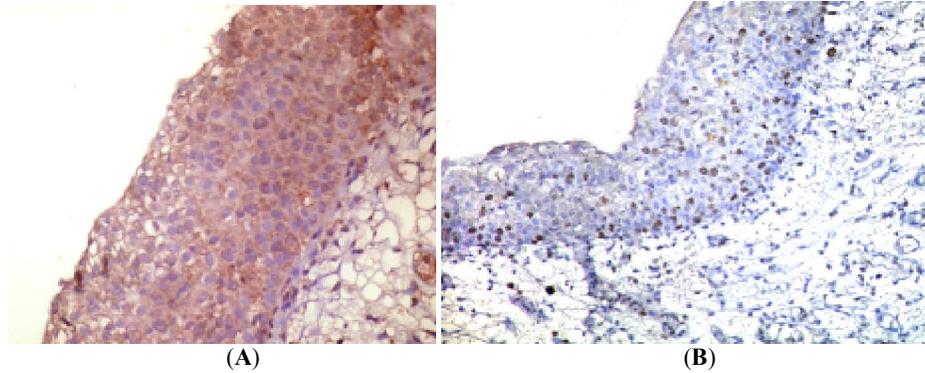


Fig. (4): AUS: (A) Abnormal CK20 expression with diffuse, strong full-thickness staining X400. (B) Moderate P53 expression X 200

4. Discussion

Histopathological diagnosis of CIS is dependent on cytological features such as nuclear enlargement, hyperchromatasia, variation in shape, size at nuclear and cellular level and increased mitotic activity. But its diagnosis may be difficult to be differentiated in cases with reactive atypia, especially in certain morphologic variants of CIS including pagetoid CIS and clinging CIS, as well as dysplastic lesions showing appreciable cytological atypia (not severe enough to diagnose CIS), may cause diagnostic troubles. The differentiation of UD and CIS from reactive atypia is critical because it has both therapeutic and prognostic importance.^{8,19} To overcome this issue, earlier studies had proposed the use of CK20, CD44, Ki67, p53, and recently p16 and CK5/6 immunostains as ancillary aids.^{11,20}

Specific markers of UD/CIS would be of great value to surgical pathologists as adjuncts to morphology in this setting.²¹

In our study, the normal urothelium showed patchy cytoplasmic staining in the superficial (umbrella) cell layer by CK20 in all cases, whereas P53 nuclear immunoreactivity varied from none to focal. CK5/6 showed staining of only the basal layer. These results are consistent with the previous studies.²⁰

Regarding reactive urothelial atypia, 95% of cases showed CK20 expression only in the umbrella cell layer similar to normal urothelium. However, one case showed focal expression, but this was considered as non-specific because of its benign morphology with positivity for CK5/6 and absent P53. This result is close to the result of Kunju *et al.*¹⁹ who had (96%) of reactive urothelial atypia negative with CK20. Previous studies were done by McKenney *et al* and Mallofre *et al*; they reported that 100% of reactive urothelium was negative.^{15,22}

Our study demonstrated that diffuse full thickness CK5/6 expression in RA differentiates these benign lesions from UD and CIS. The advantage of

CK5/6 over other markers is its availability, its distinct staining pattern. These findings agree with the results of Edgecombe *et al.*²⁰

P53 staining pattern in most cases of reactive atypia varied from absent to patchy and weak nuclear reactivity, predominantly in the basal cell layer, similar to previous studies.^{15,23} These immunoreactivity pattern of reactive urothelial atypia confirmed that it is not a premalignant lesion and is placed under benign urothelial abnormalities.

The main value of a panel of immunostains would be its ability to diagnose cases of AUS. Based on our results, a panel of CK20, P53 and CK5/6 may be used to resolve cases of AUS. AUS is not a diagnostic entity but it is a descriptive term used in diagnostically difficult cases. In the present study, 3 cases of AUS could be categorized as reactive (CK5/6 positive, both CK20 and P53 negative). We confirmed these cases after the first follow up. The remaining three cases of AUS were thought to be UD; diagnosis of CIS was confirmed in two cases after the second follow up and the third cases showed mild UD after the first follow up. And the pathologist became assured in histopathological examination. Our results are consistent with the previous study by Kunju *et al.*¹⁹, who depended on a panel of 2 markers (CK20 and KI67) to resolve cases of (AUS). But its differ from the results of Cheng *et al.*⁸ who found that none of their 35 patients with the diagnosis of (AUS) developed urothelial carcinoma, carcinoma in situ or dysplasia. This difference could be attributed to that; we segregated cases of AUS according to immunohistochemical staining pattern into reactive and dysplasia, but Chang and colleagues depended only on histopathologic features and follow up. Cytokeratin 20 which was first characterized by Moll *et al.*²⁴ is not expressed in normal urothelium, except by occasional umbrella cells and intermediate cells. CK20 expression in urothelial carcinoma tissue correlated with tumor grade and also predicted early recurrence.²⁵

To our knowledge, no previous study in the literature has evaluated the utility of the immunohistochemical panel (CK20, P53 and CK5/6) in urothelial dysplasia as a separate group. In our study, CK 20 reactivity in dysplasia cases was positive in (81.8%), and this is consistent with Yildiz *et al.*²³ This result reflects that; CK20 may be a reliable and relatively diagnostic marker of UD in conjunction with morphology.

Dysplasia showed CK20 expression nearly similar to CIS (81.8%, 84.6% respectively). However P53 expression in dysplastic group was less than CIS (54.5%, 69.2% respectively). CK5/6 was negative in all cases of dysplasia and CIS. So; it is recommended that severe dysplasia and CIS may be combined into a single category. McKenney *et al.* and Kunju *et al.*^{15, 19} didn't segregate dysplastic urothelial lesions into dysplasia and CIS. Histological criteria for distinguishing severe dysplasia from CIS are unreliable that the urothelial dysplasia is a significant risk factor for urothelial carcinoma. Close clinical follow up, and regular cystoscopic examinations are recommended for patients with urothelial dysplasia.

CIS of the urinary bladder is a "real" malignant lesion, in contrast to other in situ lesions, like testicular CIS and prostatic intraepithelial neoplasia (PIN), which are considered precursors of malignancy. Subsequently the importance of diagnosis and treatment of CIS is obvious, since lack of treatment indicates a more than 50% 5-year progression rate and higher recurrence rate.²⁶

CIS is a dangerous disease, with a small gap between progression and successful treatment. Early detection of CIS would help in the reduction of bladder cancer incidence, morbidity and mortality.² In our study, 84.6% CIS cases had full thickness CK20 positivity. However, two cases showed positivity only in the upper third of the urothelium accompanied by positive P53 and also were negative for CK5/6. CK20 and P53 promising to be reliable diagnostic marker of CIS in conjunction with morphology. Strong P53 was observed in 69.2%. While, four cases with weak P53 had positive CK 20 and negative CK5/6. Our results are similar to previous studies done by^{15, 19, 20, 22, 23}.

An immunohistochemical profile characteristic of CIS (CK20 and P53 over-expression and lack of CK5/6 reactivity in the malignant cells) adds further support to the initial diagnosis of CIS based solely on morphology. In contrast, diffuse, full thickness staining with CK5/6 together with a lack of CK20 and P53 would favor a reactive process. Based on our findings, this immunohistochemical panel may be of great value (*p* value <0.001) in special diagnostic settings as; cases in which the diagnosis of CIS is strongly clinically favored but the cytological features are equivocal, diagnosis of primary CIS and

confirming unusual morphologic presentations of CIS such as pagetoid or undermining CIS.

Conclusion:

CK20, CK5/6 and P53 are promising to be reliable diagnostic markers of UD and CIS in conjunction with morphological changes especially in cases of diagnostically challenging biopsies; and help to reach a definite diagnosis in AUS cases. Ck20 only cannot differentiate between UD and CIS, in biopsies of flat intraurothelial lesions with atypia, further studies with larger series of AUS cases and long period of follow up would be required to confirm these findings.

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Conflict of interest

None

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References

1. Jemal A, Ward E, Melissa M, Bray F, Forman D, Ferlay J: Cancer statistics, CA Cancer J Clin 2011 61, 2, p. 69–90.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers CD and Parkin D: Globocan 2008, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 10. Lyon, France: International Agency for Research on Cancer; Year. Available at: <http://globocan.iarc.fr>. 2010. Last accessed 8/17/2010.
3. Cheng L, Lopez-Beltran A, MacLennan GT, Montironi R and Bostwick DG: Neoplasms of the urinary bladder. In: Bostwick DG, Cheng L, editors. Urologic surgical pathology. 2nd ed. Philadelphia: Elsevier/Mosby; 2008. p. 259-352
4. Eble JN, Sauter G, Epstein JI and Sesterhenn IA: World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs Lyon: IARC Press, 2004.
5. [Seisen](#) HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed?term=Seisen%20T%5BAuthor%5D&cauthor=true&cauthor_uid=24176406"__T and Rouprêt M. Transurethral bladder resection of non muscle invasive bladder cancer with Hexvix ®Prog Urol. 2013; 23(14):1177-80.

6. Epstein, J I; Amin, M B and Reuter, V E. Flat Urothelial Lesions. In Weinberg W R, Consoli L, Geffner J(ed): Bladder Biopsy Interpretation, 1st ed. Philadelphia, Lippincott Williams & Wilkins (2004); 12-34.r
7. Holmång S, Ströck V. Should follow-up cystoscopy in bacillus Calmette-Guérin-treated patients continue after five tumour-free years? *Eur Urol* 2012; 61(3):503-7.
8. Cheng L, Cheville JC, Leibovich BC, Weaver AL, Egan KS, Spotts BE *et al.*: Survival of patients with carcinoma in situ of the urinary bladder. *Cancer* 1999; 85: 2469–74.
9. Cheng L, Lopez-Beltran A, David G and Bostwick DG: Bladder pathology chapter, Flat Urothelial Lesions with Atypia and Urothelial Dysplasia. Published Online: 9 JUL 2012, ISBN 978-0-470-57108-8
10. papillary lesions and conditions of the urinary bladder: an update based on the Ancona International Consultation. *Virchows Arch* 2002; 440: 3–11.
11. Hodges KB, Lopez BA, Davidson DD: Urothelial dysplasia and other flat lesions of the urinary bladder: clinicopathologic and molecular features. *Hum Pathol* 2010; 41:155–62.
12. Cheng L, Zhang S, MacLennan GT, Williamson SR, Lopez-Beltran A and Montironi R: Bladder cancer: translating molecular genetic insights into clinical practice. *Hum Pathol* 2011; 42: 455–81.
13. Takenaka A, Yamada Y, Miyake H, Hara I and Fujisawa M: Clinical outcomes of bacillus Calmette-Guérin instillation therapy for carcinoma insitu of urinary bladder. *Int J Urol* 2008; 15: 309–13.
14. Desai S, Lim SD, Jimenez RE, Chun T, Keane TE, McKinney JK *et al.*: Relationship of Cytokeratin 20 and CD44 protein expression with WHO/ISUP grade in pTa and pT1 papillary urothelial neoplasia. *Mod Pathol* 2000; 13: 1315–23.
15. McKenney JK, Desai S, Cohen C and Amin MB: Discriminatory immunohistochemical staining of urothelial carcinoma in situ and non-neoplastic urothelium: an analysis of cytokeratin 20, p53, and CD44 antigens. *Am J Surg Pathol* 2001; 25:1074-8.
16. Moll R, Franke WW, Schiller DL Geiger B and Krepler R: The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 1982; 31: 11–24.
17. Yin H, He Q, Li T, Leong AS: Cytokeratin 20 and Ki-67 to distinguish carcinoma in situ from flat non-neoplastic urothelium. *Appl Immunohistochem Mol Morphol* 2006; 14: 260–5.
18. Jung S, Wu C, Eslami Z, Tanguay S , Aprikian A, Kassouf W *et al.*: The role of immunohistochemistry in the diagnosis of flat urothelial lesions: a study using CK20, CK5/6, P53, Cd138, and Her2/Neu *Ann Diagn Pathol*. 2014 Feb;18(1):27-32.
19. Kunju LP, Lee CT, Montie J and Shah RB: Utility of cytokeratin 20 and Ki-67 as markers of urothelial dysplasia. *Pathol Int*.2005; 55: 248–254
20. Edgecombe A, Nguyen BN, Djordjevic B, Belanger EC and Mai KT: Utility of cytokeratin 5/6, cytokeratin 20, and p16 in the diagnosis of reactive urothelial atypia and noninvasive component of urothelial neoplasia. *Appl Immunohistochem Mol Morphol* 2012; 20: 264–71.
21. Kim JC and Steinberg GD: The limits of bacillus Calmette-Guerin for carcinoma in situ of the bladder. *J Urol* 2001; 165: 745–56.
22. Mallofré C, Castillo M, Morente V and Solé M: Immunohistochemical Expression of CK20, p53, and Ki-67 as Objective Markers of Urothelial Dysplasia *Mod Pathol* 2003; 16: 187–91
23. Yildiz ZI, Recavarren R, Armah HB, Bastacky S, Dhir R and Parwani AV: Utility of a dual immunostain cocktail comprising of p53 and CK20 to aid in the diagnosis of non-neoplastic and neoplastic bladder biopsies. *Diagn Pathol* 2009; 4: 35.
24. Moll R, Lowe A, Laufer J and Franke WW: Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. *Am J Pathol* 1992; 140: 427-47.
25. Harnden P, Eardley I, Joyce AD and Southgate J: Cytokeratin 20 as an objective marker of urothelial dysplasia, *Br J Urol* 1996; 78: 870–875.
26. Wolf H, Melsen F, Pedersen SE and Nielsen KT: Natural history of carcinoma in situ of the urinary bladder. *Scand J Urol Nephrol Suppl* 1994; 157: 147–51.