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Original Article

Role of CK20 immunocytochemistry as an adjuvant to urine cytology in the detection of urothelial malignancy

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Abstract

Introduction: *Urine cytology has been a non-invasive method of choice for detecting urothelial carcinomas but it has several drawbacks including low sensitivity for low grade urothelial lesions, morphology being easily affected by infection, instrumentation, surgery, chemo or immune therapy. Additional screening tests with high sensitivity and specificity for urothelial tumours of all grades are indicated to help improve the diagnostic ability of urine cytology as well as to reduce the need for frequent cystoscopies, especially in those with low-risk disease.*

Objective: *We studied the role of CK20 immunocytochemistry (CK20 ICC) and compare the results with efficacy of cytology in detecting urothelial carcinomas.*

Material and Methods: *We studied 160 cytospin centrifuged smears of urine cytology stained with hematoxylin and eosin and were reported according to the Paris system. Cell blocks were prepared in each case and stained with CK 20 monoclonal antibodies.*

Results: *Sensitivity, specificity, positive predictive value and negative predictive value of urine cytology was 78.72%, 98.05%, 94.87% and 91.15% respectively while that of CK20 ICC was 85.11%, 94.29%, 86.96% and 93.40% respectively.*

Conclusion: *CK20 ICC is more sensitive than urine cytology for detection of all grades and stages of bladder cancer. It is recommended as useful adjuvant to urine cytology in controversial cases.*

Keywords: *voided urine cytology, The Paris System, CK 20 immunocytochemistry*.

Introduction

Urinary bladder carcinoma is the commonest urological malignancy in India[1]. However, it is the peculiar biological behavior of long natural history of these tumors spanning upto15-20 years which is more significant than its overall mortality or incidence. Majority of the bladder tumors (70%) are superficial and curable but unfortunately 50-70% of these patients present with recurrence, up to a third of which are of higher grade and/or stage[2].

Urine cytology is an accepted diagnostic aid for detection of urothelial carcinoma and for follow up of patients who have the disease for recurrence. Numerous studies conducted over the century have established undisputed high sensitivity (50-85%) for high grade urothelial lesions but much lower sensitivity anywhere between 10 to 43.6% in patients with low grade lesions[2].

Difficulties and challenges of urine cytology have led to development of various biomarkers to improve its sensitivity. One such technique is the use of monoclonal cytokeratin 20 (CK 20) antibodies for identification of malignant cells isolated from voided urine. International Society of Urologic Pathology (ISUP) conference on "Best practice recommendations in the application of immunohistochemistry in bladder lesions" have established panel of CK20/p53/CD44 as a gold standard for differential diagnosis in histology[3]. But there are limited studies studying CK20 expression in urine cytology smears.

The present study is conducted to analyze the role of CK20 immunocytochemistry (CK20 ICC) in urine samples sent for cytology and compare the results with the efficacy of cytology in detecting urothelial carcinomas.

Material and Methods

In this prospective study, 160 cytospin centrifuged smears of voided urine cytology were studied over a period of two years. It included patients attending urology OPD being evaluated for unexplained hematuria, lower urinary tract symptoms, patients with equivocal cystoscopy or positive radiological findings and high risk group (>40years, smoker, analgesic abuse). It was also done for detection of recurrence in patients treated for bladder cancer. Smears were stained with hematoxylin and eosin. They were reported according to the criteria defined in The Paris System for reporting urine cytology.[2]

The cell pellet remaining after preparing smears was processed into cell block by mixing it with AAF fixative which consists of 95% ethyl alcohol – 34ml, formalin – 4ml and glacial acetic acid – 2ml. Then after centrifuging for 10 minutes at 2500 rpm, the cell button was re-suspended in AAF fixative and after 4-6 hours centrifuged again for 10 minutes at 3000 rpm, supernatant was discarded and formalin was added. The tube was kept for 4 — 6 hours. Cell button was then scraped and wrapped in filter paper and processed in tissue processor. Paraffin embedded sections on polylysine coated slides of the cell blocks so prepared were used for immunostaining with CK 20 monoclonal antibodies. Diagnosis of urothelial carcinoma was given if >5% or more cells showed cytoplasmic positivity.

The results of urine cytology and CK20 were compared with histopathology which was considered as gold standard and only end point variable available to calculate sensitivity and specificity for each test. Histopathological diagnosis was based on current 2017 WHO classification of tumours of the urinary system[4].

Results

The patients were aged between 16-95 years with mean age 53.33 years. Male: female ratio was 7:1. Total number of patients evaluated for hematuria (Macroscopic/ microscopic) were 125/160(78.13 %), for lower urinary tract symptoms were 19/160(11.87 %) and 16/160 (10.0 %) were follow up cases of urothelial carcinoma investigated for recurrence.

On cytological examination, we regarded 8 cases as inadequate in accordance with The Paris System. It included cases with only squamous cells and no urothelial cells, <5 well preserved well visualized urothelial cells due to obscuring neutrophils and extensive degeneration impairing evaluation of atypia. 152 samples (95.0%) were regarded as adequate for reporting.

Following The Paris System of reporting for urine cytology out of 152 cases, 65.79% (100/152) were reported as negative for high grade urothelial carcinoma (NHGUC), 8.55% (13/152) were atypical urothelial cells (AUC), 4.61% (7/152)

were suspicious for high grade urothelial carcinoma (SHGUC), 20.39% (31/152) were positive for high grade urothelial carcinoma (HGUC) and only a single case (0.66%) was reported as positive for low grade urothelial neoplasm (LGUN). CK20 antibody was applied on all 152 cases. Using 5% of stained cells as the threshold for positive diagnosis, 30.26% cases (46/152) was positive. [Figure 1]

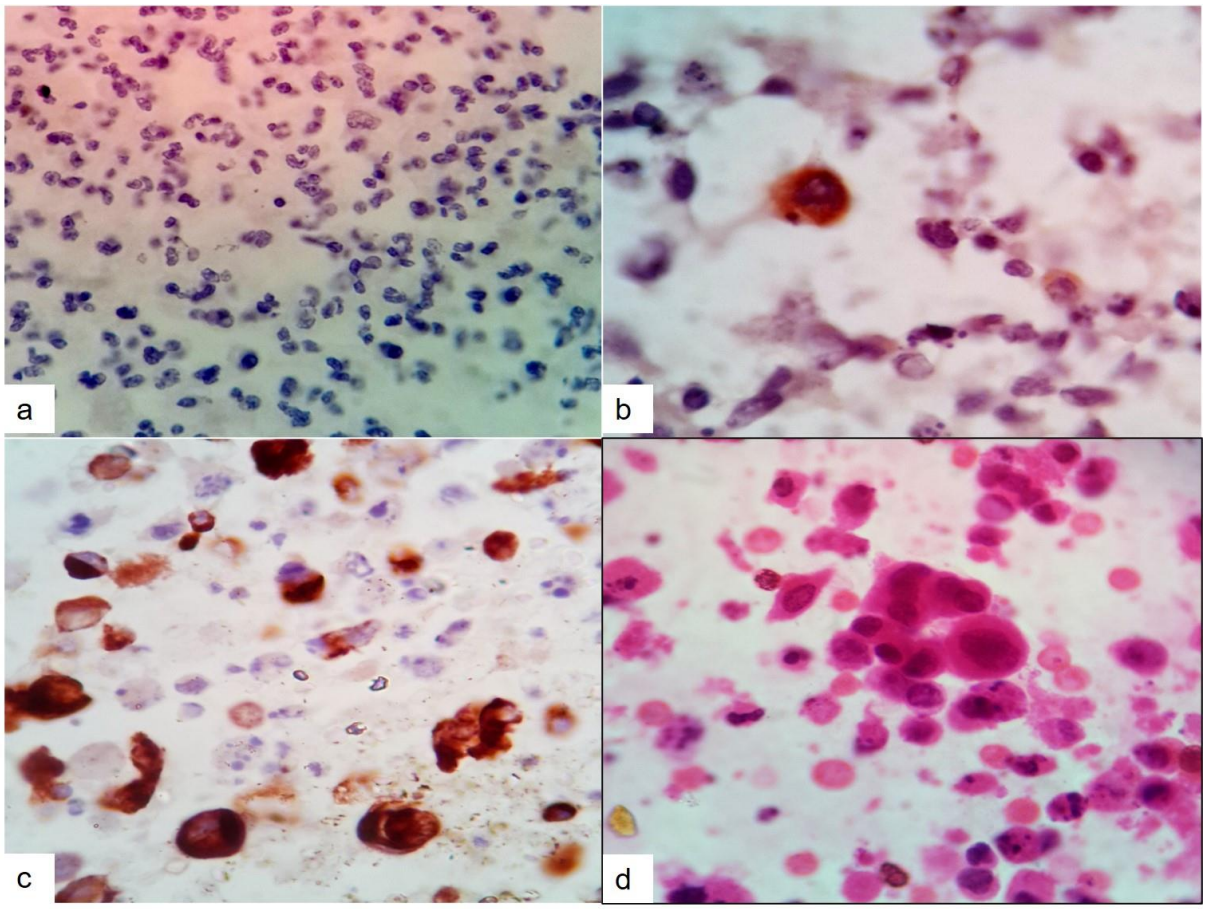

Figure 1: A- negative for CK20 ICC. B, C- positive for CK20 ICC. D- high grade urothelial carcinoma on urine cytology in positive case.

There were 47 histologically diagnosed cases of urothelial carcinoma. It included 37 cases of high grade urothelial carcinoma (invasive urothelial carcinoma+ noninvasive high grade papillary urothelial carcinoma) and 10 cases of low grade urothelial carcinoma.

Diagnostic categories that were considered as positive urine cytology were SHGUC, HGUC and LGUN since the management of all is same. Positive urine cytology was seen in 39/152 (25.66%) and biopsy proven histological urothelial carcinoma was found in 37 of these cases and in two cases there was false positive diagnosis of SHGUC. [Table1]

Table 1: Distribution of Various Categories of Urine Cytology And CK20 ICC

Histopathology	Urine Cytology	CK 20 ICC
High grade urothelial carcinoma (n=37)	NHGUC=2 AUC=4 SHGUC=3 HGUC=28	Positive=32 Negative=5
Low grade urothelial carcinoma (n=10)	NHGUC=1 AUC=3 SHGUC=2 HGUC=1 LGUN= 1	Positive=8 Negative=2

Rate of detecting urothelial carcinoma on biopsy after giving the diagnosis of atypia on urine cytology was 61.53% while it was 71.43% in suspicious cases.

Urine cytology had sensitivity of 78.72% for detecting urothelial carcinomas which increases upto 83.78% for high grade urothelial carcinomas and is only 60.0% for low grade urothelial carcinomas. However, specificity is 98.09%, positive predictive value is 94.87% and negative predictive value is 91.15% for detecting urothelial carcinomas.

Out of 37 cases of high grade urothelial carcinomas, 32 were positive for CK20 while out of 10 cases of low grade urothelial carcinomas, 8 were positive for CK20 thus showed sensitivity and specificity of 85.11% (confidence interval 74.3%-93.9) and 94.29%(confidence interval 87.0%-96.8%) respectively for detecting urothelial carcinomas. Positive predictive value was 86.96% and negative predictive value was 93.40%. Its sensitivity for high grade urothelial tumours was 86.48% and for low grade urothelial tumours was 80%. Out of 7 tumors missed by CK20 ICC, 2 were low grade and 5 were high grade malignancies. Results were not affected by the presence of inflammatory cells in the specimen.

Combined sensitivity of urine cytology with CK20 immunocytochemistry is 91.48% (confidence interval 77.8% - 95.5%) and specificity is 95.23% (confidence interval 90.5%-98.5%) for diagnosing urothelial carcinomas.

Table 2: Diagnostic accuracy of urine cytology and CK20 ICC for detecting urothelial carcinoma

	Urine cytology	CK20 ICC	Urine cytology + CK20 ICC combined	Urine cytology for high grade urothelial carcinoma	CK20 ICC for high grade urothelial carcinoma	Urine cytology for low grade urothelial carcinoma	CK20 ICC for low grade urothelial carcinoma
Sensitivity	78.72%	85.11%	91.48%	83.78%	86.48%	60.0%	80%
Specificity	98.09%	94.29%	95.23%	93.04%	87.82%	76.76%	73.23%
Positive predictive value	94.87%	86.96%	89.58%	79.48%	69.56%	15.38%	17.39%
Negative predictive value	91.15%	93.40%	96.15%	94.69%	95.28%	96.46%	98.11%

Discussion

Urine cytology is a simple, noninvasive and cost-effective screening tool for the diagnosis of urothelial malignancies. While a number of urinary biomarkers such as BTA stat, BTA TRAK, NMP22, and Urovysion TM have been approved by the Food and Drug Administration for either diagnosis or follow-up but they are expensive, need technical expertise and their availability in developing countries, including India, is limited.[2] CK20 expression is limited to umbrella cells in normal urothelium, but extends to deeper layers in malignancy. Hence, it is a potential candidate for the diagnosis of urothelial malignancy, especially

in LGUC with subtle cytological features with added advantages of relatively low cost and wide applicability including in resource limited settings. In our study, we found sensitivity and specificity of urine cytology was 78.72% and 98.09% respectively for diagnosing urothelial carcinomas. Other studies reported sensitivity from 29.6% to 76.2% and specificity 66.7% to 100.0%. [5-10] We reported a higher sensitivity of 78.72% because of following the stringent and well-defined criteria of high-grade urothelial lesions detected on urine cytology using The Paris System. Still, it had limitations in diagnosing low grade lesions.

There are only few studies using CK20 in cytology for detecting urothelial carcinoma. Usually, immunocytochemical slides are often characterized as positive even in the presence of a few stained cells. However, in the case of CK-20, such an approach is rendered unacceptable, because, apart from the neoplastic urothelial cells, their differentiated superficial counterparts known as "umbrella" cells also express low levels of CK20. Hence, to reduce the risk of false-positive results, and in accordance with previous studies, we set the threshold for a positive diagnosis at 5% of stained cells, recognizing that it may affect the sensitivity of the assay.[12]

On applying CK 20 immucocytochemistry on cell blocks, we found sensitivity of 85.11% and specificity of 94.29% for diagnosing urothelial carcinomas. Other studies showed sensitivity from 65.3% to 94.4% while specificity from 67.0% to 100%.[5-8,10,12-13] Variable chemical nature of urine and poor preservation of malignant cells which can affect the staining have significant impact on diagnostic accuracy. [Table 3]

Table 3: Comparison of diagnostic accuracy of CK20 ICC in urine cytology in various studies

STUDY	YEAR	SENSITIVITY	SPECIFICITY	PPV	NPV
Golijanim et al. [5]	2000	81.6%	77%	78%	80%
Lin S et al.[12]	2001	94.4%	80.5%	-	-
Melissougos et al.[6]	2005	65.3%	90.9%	92.2%	61.5%
Bhatia et al.[13]	2007	86.0%	100%	-	-
Xiang yong gu et al.[7]	2008	89.1%	80.0%	92.6%	53.3%
Souyer et al.[8]	2009	70.4%	83.3%	86.3%	65.2%
Vinod kumar arora et al.[10]	2011	70.4%	71.4%	90.5%	38.5%
Wadhwa et al.[15]	2017	88.1%	95.0%	97.4%	79.2%
Present study	2021	85.11%	94.29%	86.96%	93.40%

Our study showed better sensitivity of CK20 ICC (85.11%) over urine cytology (78.72%) while urine cytology has better specificity (98.09%). It was similar to the results of Golijanim et al. and Xiao- yong et al.[5,7] Melissourgos et al. found CK20 immunocytochemistry to have both improved sensitivity (65.3%) and specificity (90.9%) over urine cytology (54.2%; 86.4% respectively) while Souyer et al. found lower sensitivity (70.4%) of CK20 immunocytochemistry with better specificity (83.3%) than urine cytology (75.9%;66.7%).[5-8] Vinod et al. found lower sensitivity and specificity of CK20 ICC than urine cytology to detect urothelial malignancies.[10] This was because their study was retrospective and used archival material. Non coated slides of urine cytology were kept in xylene for removal of cover slip, then destained and was used for immunocytochemistry. This led to the cell loss which might have resulted in false negativity. In our study, CK 20 immunocytochemistry showed improved sensitivity over urine cytology for both low grade and high grade urothelial malignancies. Other studies showed similar results. Klein et al and Golijanin et al. found sensitivity of CK20 ICC

was much better than that of cytology for every grade and stage of bladder cancer.[5,14] Sensitivity of combination of both tests was 91.48% while specificity was 95.23% implying an improved diagnostic performance than either of them used alone. Similar results were reported by Souyer et al. with combination showing sensitivity of 77.8%.[8]

Lin et al. found CK20 to be excellent marker for detecting urothelial carcinoma in atypical urine cytology. He concluded CK20 can be used to triage atypical urine cytology into low risk and high risk groups. Patients with subsequent positive cytology after initial reporting as atypia had 94.4% positivity of CK20 while others had only 27.3%.[12] We applied it on 11 cases of atypia, two had inadequate material to prepare cell block. It was positive in 9 cases while two were negative. Out of those 09 cases, 8 had urothelial carcinoma i.e. detection rate of 88.8% and one had metaplasia. So CK20 is a useful marker for detecting urothelial carcinoma in atypical cases of urine cytology. It was also found in the study of Bhatia et al.[13]

Cytology and cystoscopy have been used as initial diagnostic tests for patients suspicious for bladder cancer or for the surveillance of patients for tumor recurrence. Cystoscopy is highly sensitive but may fail to identify flat tumors such as carcinoma in situ. Also, it is invasive and costly for patients needing frequent monitoring. Conversely, urinary cytology is noninvasive and highly specific but has poor sensitivity for low grade lesions.

We studied role of CK 20 ICC as a diagnostic tool for detecting urothelial carcinoma. It is more sensitive than urine cytology and also has an added advantage of easier interpretation over urine cytology since the only criterion of a positive result is the presence of a significant number of cells (5%) with the typical red brown color in cytoplasm. It was not affected by the presence of obscuring inflammatory cells or RBC in the sample but trapping of background staining along with overlapping of cells can cause false positive readings by inexperienced pathologist.

Conclusion

CK20 ICC is more sensitive than cytology for detection of all grades and stages of bladder cancer. It is simpler to interpret than Paris system in atypical cases detecting >5% cells with red brown cytoplasm. However, CK 20 immunocytology may sometimes miss high grade transitional cell carcinoma and, therefore, cannot be used as a routine substitute for cytology. Combining CK20 ICC with urine cytology is recommended in ambiguous cases to improve the detection of urothelial carcinomas in voided urine cytology.

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