Cytological and Morphometric Study of Urinary Epithelial Cells with Histopathological Correlation

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Abstract This study has been done to assess the utility and accuracy of urinary cytology and morphometric study of exfoliated cells in early detection and follow-up of urothelial neoplasms and thereby help to reduce the disease-related mortality and morbidity. A total 100 patients with urinary symptoms were studied by cytological examination of urine along with morphometric analysis of suspicious epithelial cells. Immunostaining to detect CK-20 expression and p53 over expression was done in smears showing atypical cells. Histopathological confirmation was done in cases which were suspicious on cystoscopy. *P* value was determined by using unpaired *t*-test. Statistically significant difference was found between neoplastic and non-neoplastic lesions of the urinary bladder as far as morphometry is concerned. Urinary cytology along with morphometry is an important tool in early detection of urothelial neoplasms. It is also helpful to find out the recurrences during post-operative follow-up period. Overexpression of CK-20 and p53 immunostain in cytology can act as an adjunct to the cytological diagnosis.

Keywords Urine cytology · Morphometry · CK-20 · p53

Introduction

Neoplasms of the urinary tract from renal pelvis to urinary bladder may be primary or secondary. Benign papilloma, transitional cell carcinoma (TCC), squamous cell carcinoma, adenocarcinoma, embryonal rhabdomyosarcoma, etc., are primary neoplasms, and secondaries arise from breast, sigmoid colon, prostate, uterus, ovary, etc. TCC comprises approximately 90 % of all primary tumors. Cytological examination of urinary sediment is now well established as a part of routine investigation of patients with hematuria, symptoms of prostatism, and suspected urinary tract neoplasia [1].

Cytological examination of urine can detect most high-grade bladder carcinoma and useful for carcinoma in situ, which is difficult to diagnose cystoscopically. However, it has limited success in diagnosis of low-grade urothelial carcinoma in which case morphometric study is a useful tool [2]. Detection of CK-20 and overexpression of p53 gene have been advocated as an adjunct to the cytological diagnosis. CK-20 expression is commonly seen in neoplastic cells but not in normal urothelial cells in urine cytology [1].

The greatest value of urinary cytology is in the following situations:

1. Tumor associated with extensive chronic inflammation
2. Carcinoma in situ
3. Carcinoma hidden in bladder diverticulum [1]

False-positive cytology is most often due to cellular atypia associated with calculi, inflammation, and intravesical chemotherapy. Features favoring neoplasia includes greater cellularity

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in centrifuge deposit with more cell dissociation, nuclear pleomorphism, and irregular chromatin pattern. So, urinary cytology and morphometric study is an important noninvasive method for screening, early diagnosis, and follow-up of patients with malignant neoplasms of urinary tract [2].

Aims and Objectives

1. Study of urinary cytology with special reference to neoplastic lesions of urinary tract
2. Morphometric analysis of suspicious cells to increase the accuracy
3. Role of cytokeratin 20 (CK-20) expression and P53 overexpression in early detection of urothelial malignancy in voided urine specimens

Materials and Methods

This study was carried out in the department of pathology at our hospital over a period of 2 years (from June 2007 to June 2009). A total 100 voided urine specimens were collected from the patients attending urology OPD with complaint of hematuria, symptoms of prostatism, and abdominal pain. Morning urine specimens were collected and centrifuged, and two slides were prepared for each case. One May-Grunwald-Giemsa and one Papanicolaou stained smears were examined. In 46 cases, epithelial cells were found in urine. Morphometry of the cells in these 46 cases was done by using ocular and stage micrometers. Urine smears of 29 cases (4 cases of suspicious cytology, 16 cases of low-grade TCC, and 9 cases of high-grade TCC as provisionally diagnosed on cytology with morphometry) were taken for immunostain by monoclonal CK-20 antibody and P53. We classified the expression pattern of CK20 as negative or positive at the time of

initial diagnosis. The P53 expression was analyzed by determining the percent positivity of nuclear stain after counting 50 cells. Urine samples from 10 volunteer healthy donors were used as control for urine cytology, and bladder washings from 5 biopsy-proven transitional cell carcinoma patients were taken for positive control of immunostain. Histopathological confirmation was done in cases which were cystoscopically positive for the suspicious lesion.

Results

In the 2-year study period, urine specimens were collected from total 100 patients. Cytological examination of May-Grunwald-Giemsa and Papanicolaou stained smears from these 100 cases revealed 17 cases of inflammatory smear associated with reactive epithelial cells, 4 cases suspicious of malignancy, 25 cases positive for malignancy out of which 16 cases were diagnosed as low-grade TCC and 9 cases as high-grade TCC by morphometry (Tables 1 and 2). Cellularity was scanty in rest of 54 cases. The detailed cytological features such as cellularity, cell clustering, papillae, necrosis, apoptosis, nucleocytoplasmic (NC) ratio, nuclear pleomorphism, nuclear margin, hyperchromasia, and chromatin abnormalities were observed.

In all these cytologically positive cases, morphometric analysis was performed in terms of cell diameter, nuclear diameter, and NC ratio (Table 2). Urine samples from 10 volunteer healthy donors were used as control for urine cytology. Urine smears of 29 cases (4 cases of suspicious cytology, 16 cases of low-grade TCC, and 9 cases of high-grade TCC as provisionally diagnosed on cytology along with morphometry) were selected for immunostain by monoclonal CK-20 antibody and P53.

CK-20 expression was positive in all cytological smears taken from 16 low-grade and 9 high-grade TCC samples,

Table 1 Distribution of cases according to cytological and histopathological diagnoses

Cytological diagnosis					Histological diagnosis	
Inflammatory smear (17 cases)	Suspicious cytology (4 cases)	Low-grade TCC (16 cases)	High-grade TCC (9 cases)	Squamous cell carcinoma (0 cases)	Category	No of cases
0	2	0	0	0	(i) Papillary urothelial neoplasms of low malignant potential	2
0	0	14	0	0	(ii) LGTCC	14
0	0	2	8	0	(iii) HGTCC	10
0	0	0	1	0	(iv) Squamous cell carcinoma	1
0	1	0	0	0	(vi) Chronic cystitis	1
17	1	0	0	0	(vii) No visible lesion	18

LGTCC, low-grade TCC; HGTCC, high-grade TCC

Table 2 Results of morphometric study in different categories from Papanicolaou-stained cytological smear

Diagnosis	Mean cell diameter ($\mu\text{m}\pm 2\text{SD}$)	Mean cell area ($\mu\text{m}^2\pm 2\text{SD}$)	Mean nuclear diameter ($\mu\text{m}\pm 2\text{SD}$)	Mean nuclear area ($\mu\text{m}^2\pm 2\text{SD}$)	Mean NC ratio
Normal Transitional cell	17.26 $\pm$ 0.75	232 $\pm$ 23.5	6.5 $\pm$ 0.11	30.35 $\pm$ 2.15	0.13
Inflammatory smear	15.99 $\pm$ 1.53	200 $\pm$ 38.38	6.29 $\pm$ 0.72	30.76 $\pm$ 6.98	0.15
Suspicious for malignancy	15.55 $\pm$ 0.96	188 $\pm$ 24.95	8.82 $\pm$ 0.59	60 $\pm$ 6.93	0.31
Transitional cell carcinoma (low grade)	13.51 $\pm$ 2.056	142 $\pm$ 42.95	10.05 $\pm$ 1.32	79.25 $\pm$ 20.49	0.55
Transitional cell carcinoma (high grade)	14.05 $\pm$ 0.85	153 $\pm$ 17.15	12.14 $\pm$ 1.01	116 $\pm$ 17.52	0.75

whereas 2 cases were positive for CK-20 expression out of 4 cases of suspicious cytological smears. Overexpression of p-53 in cytological smears (>60 % cells show nuclear positivity) was found in all 9 high-grade and in 2 low-grade TCC cases (Table 3). Histopathological confirmation was done in cases which were cystoscopically positive for any suspicious lesion (Table 1). Biopsy specimens were obtained in 28 cases only; in rest of 18 cases no suspicious lesion was seen on cystoscopic examination.

A grand chart was produced tabulating cytological diagnosis, results of morphometric analysis, and histopathological diagnosis where biopsy available. The results obtained were analyzed.

Discussion

In the 2-year study period, we had examined urine specimens from total 100 patients presenting with hematuria, dysuria, or flank pain. In case of nonneoplastic inflammatory lesions the male-to-female ratio was 2.4:1, and in neoplastic lesions the ratio was 7.33:1. Cytological examination from these 100 cases had revealed 17 cases (17 %) of inflammatory smear, 4 cases (4 %) suspicious of malignant lesion, 25 cases (25 %) of urothelial carcinoma out of which 9 cases (9 %) were diagnosed as high-grade TCC and 16 cases (16 %) as low-grade TCC (Table 1). In the rest of 54 cases (54 %), cellularity of the urinary smears was very scanty and negative for inflammation or any atypical cell.

In this, 17 cases were diagnosed as inflammatory lesions—none of which could be confirmed histologically as no suspicious lesion was seen on cystoscopic examination. Twenty-five cases (25 %) were diagnosed as malignant urothelial

lesions on cytology and morphometry, all of which were confirmed by histopathology (Table 1). This was comparable with the study done by Highman who found 20 % suspicious cases on cytology [3]. Most of patients were male and the male-to-female ratio was 7.33:1. The malignant lesions were mainly found in elderly patients.

We found 16 cases of low-grade TCC on cytology and morphometry (Fig. 1), two of which finally diagnosed as high-grade urothelial neoplasms on histopathology (Table 1). This might be due the presence of inflammation and associated degenerative changes in the cell cytoplasm [4]. The rest of 14 cases were finally diagnosed as low-grade TCC on histology (Fig. 2). Out of 9 cases of high-grade TCC on cytology and morphometry (Fig. 3), one was diagnosed as moderately differentiated squamous cell carcinoma of urinary bladder on histopathology. Diagnosis of primary squamous cell carcinoma on cytological smear is very difficult, as the moderately differentiated carcinoma may show keratinization of cells and poorly differentiated carcinoma shows presence of giant cells only; other features are indistinguishable from high-grade TCC [5, 6]. The remaining 8 cases were corroborated with the histological diagnosis of high-grade TCC (Fig. 4). On histological examination, 3 cases of high-grade TCC show presence of deep muscle invasion.

Four cases (4 %) were categorized as suspicious for malignancy on cytology due to their increased cellularity, cell clustering without any papillae formation. In suspicious cases only the cellularity of the smear was increased, but the cells were monomorphic, found in small clusters, and had slightly increased NC ratio. The diagnosis of atypical urothelial cells in voided urine specimens was 6.9 % in a study done by Kapur et al. [7]. One case under the category of suspicious for

Table 3 The level of expression of P53 and CK20 immunostain on urine cytology smear

Cytomorphometric diagnosis	Overexpression of P53 (>60 % cells with nuclear positivity)	Immunostain for CK-20	
		Negative	Positive
Suspicious for malignant cell (4 cases)	0	2 (50 %)	2 (50 %)
Low-grade TCC (16 cases)	2	5 (31.25 %)	11 (68.75 %)
High-grade TCC (9 cases)	9	1 (11.11 %)	8 (88.88 %)

Fig. 1 Photomicrograph showing morphometry of urothelial cells in cytology of low-grade TCC (Papanicolaou×1,000)

malignancy was histologically diagnosed as chronic cystitis, and two cases were diagnosed as papillary urothelial neoplasms of low malignant potential. No visible lesion was seen on cystoscopy in another suspicious case (Table 1). This might be because small papillary tumors especially when situated in the dome or the anterior wall as well as flat dysplastic and carcinomatous lesions are difficult to identify cystoscopically. Also biopsies may miss the lesions and, even when collected from the appropriate areas, may be negative due to denudation of urothelium. Topical chemotherapy may also flatten papillary stalks rendering them invisible cystoscopically [8].

During detailed cytological observation, we found that neutrophils, lymphocytes, RBCs, and proteinaceous materials were present mainly in the inflammatory smears (17 cases) obscuring the field, and there were scattered epithelial cells singly or in tiny clusters in between them. The cell clustering was observed mainly in the neoplastic lesions. A large cluster with papillary configuration was found in low-grade urothelial carcinomas (16 cases) (Fig. 1), whereas increased cellularity with mainly scattered and a few small clusters of highly pleomorphic cells (9 cases) were found in high-grade urothelial carcinomas (Fig. 2). Papillary structures in a clean

Fig. 3 Photomicrograph showing morphometry of urothelial cells in cytology of high-grade TCC (May-Grunwald-Giemsa×1,000)

background were seen mainly in low-grade TCC. Low-grade TCC showed minimal pleomorphism of cells within a single cluster. Necrotic cell debris and inflammatory cells in the background were observed in 5 cases, all of which were high-grade urothelial carcinoma. However, diffuse background necrosis was also seen in 3 cases of inflammatory smear. Binucleate cells and cells in mitosis were seen in 4 cases of high-grade TCC. In cases of high-grade neoplasms, nuclei were large, bizarre with clump chromatin and prominent nucleoli, whereas low-grade lesions had mildly pleomorphic nuclei with homogenous chromatin pattern. Hemorrhage in the background was found in several cases of high-grade lesions, suspicious of malignant smears, and also in one inflammatory smear. Diagnostic values of the above-mentioned features in differentiating inflammatory and neoplastic lesions as well as from low-grade to high-grade urothelial neoplasms were also supported by Murphy et al., Deshpande et al., Shin et al., and Bhatia et al. [8–11].

In all these cases, morphometric analysis of the epithelial cells on cytological smears was performed in terms of cell diameter, cell area, nuclear diameter, nuclear area, and NC ratio (Table 2). In the majority of cases, the atypical cells showed marked alteration in shape and size. In this study, we

Fig. 2 Photomicrograph showing histopathology of low-grade TCC (H/E×400)

Fig. 4 Photomicrograph showing histopathology of high-grade TCC with muscle invasion (H/E×100)

found that the mean nuclear area was $30.35 \pm 2.15 \mu\text{m}^2$ for normal urothelial cells, $60 \pm 6.93 \mu\text{m}^2$ for suspicious of malignancy cases, $79.25 \pm 20.49 \mu\text{m}^2$ for low-grade TCC, and $116 \pm 17.52 \mu\text{m}^2$ for high-grade TCC cases. These results were comparable to the study by Kern et al. who had shown mean nuclear area of $36 \mu\text{m}^2$ for normal cells, $52 \mu\text{m}^2$ for benign atypical cells, $54 \mu\text{m}^2$ for cells from papillary carcinoma Grade I, $78 \mu\text{m}^2$ for papillary carcinoma Grade II, and $90 \mu\text{m}^2$ for transitional cell carcinoma Grades III and IV [12]. Nuclear abnormalities in the form of (i) nuclear pleomorphism, (ii) nuclear margin irregularity, (iii) hyperchromasia, and (iv) high NC ratio were more marked in high-grade neoplastic lesions. In this study, the NC ratio was 0.55 for low-grade TCC, 0.75 for high-grade TCC, 0.31 in suspicious cases, and 0.15 in inflammatory cases, which were also comparable with other studies. Murphy et al. showed the NC ratio <0.5 for benign conditions, and Boon et al. had found NC ratio >0.6 for high-grade neoplasms [8, 13]. Occasional mildly pleomorphic cells were observed in nonneoplastic inflammatory lesions; however, the NC ratio was low (0.15) as well as hyperchromasia and abnormal chromatin pattern were consistently absent. Boon et al. and Raab et al. had also found the similar characteristics for malignant urothelial lesions on cytology [13, 14]. In our study, nuclear features such as margin irregularity, coarse chromatin, enlargement of nuclei confirmed by morphometry, and increased NC ratio against a clean background of smears were of great help in differential diagnosis. These features were also supported in a study by Bhatia et al. [11]. There was considerable overlap in the features observed in low-grade and high-grade TCC. Some features such as necrosis, apoptosis, nuclear and chromatin changes, and prominent multiple nucleoli were more frequent in high-grade lesions which were also supported in a study by Bittinger et al. [15].

In our study, 11 (68.75 %) low-grade and 8 (88.88 %) high-grade TCC cases were positive for CK-20 on urine cytology smears which were comparable to the study done by Buchumensky et al. [16]. One high-grade TCC was CK-20 negative which on histopathology finally diagnosed as squamous cell carcinoma of bladder. Two cases of suspicious cytology were faintly positive for CK-20. These cases were diagnosed as papillary neoplasm of low malignant potential. Immunostain for p-53 had shown overexpression only in smears with high-grade TCCs and were associated with poor prognosis.

Conclusion

Despite the advancement in laboratory equipment for processing of specimens and availability of ancillary techniques for diagnosis, difficulties are still encountered in some cases. In these cases, the recognition of exact nature of the “atypical cells” in urinary cytology is very difficult. It is much difficult to distinguish between a low-grade transitional cell carcinoma

and the reactive atypia due to stone, viral infections, instrumentation, and chemotherapy. However, by using morphometry, most of cases can be categorized as low-grade or high-grade urothelial carcinoma in urinary cytology. In our study, we found four cases of suspicious cytology. On histology two of which finally diagnosed as papillary urothelial neoplasm of low malignant potential, one as chronic cystitis, and in one case no visible lesion was found on cystoscopy. In these cases, CK-20 and P-53 were helpful. Cytological examination of urine is an important tool to diagnose urinary neoplasms and to differentiate them from non-neoplastic conditions. Morphometric analysis of cytological smear and CK-20 immunostain increases the effectivity of cytology, but histopathology remains the gold standard for diagnosis.

References

1. Nelson G, Rosai J (2004) Urinary tract. Rosai and Ackerman's surgical pathology. Elsevier, New Delhi, pp 1330–1331
2. McKee G (2003) Urinary tract cytology. Diagnostic cytopathology. Elsevier, Edinburgh, pp 491–492
3. Highman WJ (1986) Transitional carcinoma of the upper urinary tract: a histological and cytological review. *J Clin Pathol* 39:297–305
4. Murata S, Kishikawa T, Isojima Y, Tsuchihashi Y, Katoh R (2004) Unusual cytologic findings in low grade papillary transitional cell carcinoma. *Acta Cytol* 48:492–496
5. Pattari SK, Dey P (2002) Urine: beyond cytology for detection of malignancy. *Diagn Cytopathol* 27:139–142
6. Beyer-Boon ME, de Voogt HJ, van der Velde EA, Brussee JA, Schaberg A (1978) The efficacy of urinary cytology in the detection of urothelial tumours sensitivity and specificity of urinary cytology. *Urol Res* 6(1):3–12
7. Kapur U, Venkataraman G, Wojcik EM (2008) Diagnostic significance of “atypia” in instrumented versus voided urine specimens. *Cancer* 114(4):270–274
8. Murphy WM (1990) Current status of urinary cytology in the evaluation of bladder neoplasms. *Hum Pathol* 21(9):886–896
9. Deshpande V, McKee GT (2005) Analysis of atypical urine cytology in a tertiary care center. *Cancer* 105(6):468–475
10. Shin BK, Lee YS, Jeong H, Lee SH, Kim H, Kim A et al (2008) Detecting malignant urothelial cells by morphometric analysis of ThinPrep® liquid-based urine cytology specimens. *Korean J Cytopathol* 19:136–143
11. Bhatia A, Dey P, Kakkar N, Srinivasan R, Nijhawan R (2006) Malignant atypical cell in urine cytology, a diagnostic dilemma. *Cytojournal* 3:28
12. Kern WH (1975) The cytology of transitional cell carcinoma of the urinary bladder. *Acta Cytol* 19(5):420–428
13. Boon ME, Kurver PH, Baak JP, Ooms EC (1981) Morphometric differences between urothelial cells in voided urine of patients with grade I and grade II bladder tumours. *J Clin Pathol* 34(6):612–615
14. Raab SS, Lenel JC, Cohen MB (1994) Low grade transitional cell carcinoma of the bladder: cytologic diagnosis by key features as identified by logistic regression analysis. *Cancer* 74(5):1621–1626
15. Bittinger A, Barth P, von Keitz A, Thomas C (1993) Urinary bladder nuclear cytology: results of karyometric investigations. *Verh Dtsch Ges Pathol* 77:209–212
16. Buchumensky V, Klein A, Zemer R, Kessler OJ, Zimlichman S, Nissenkorn I (1998) Cytokeratin 20: a new marker for early detection of bladder cell carcinoma. *J Urol* 162(2):501–502