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Is liquid-based cytology an alternative to conventional cytology for detection of malignant cells in urine of bladder cancer? Eastern Indian prospective observational study

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ABSTRACT

Objective: Conventional cytology (CC) is a widely employed biomarker for the detection of bladder cancer, but due to its decreased sensitivity, liquid-based cytology (LBC) has been studied. Despite its improved cell-free background, decreased cell degeneration, and an automated slide preparation technique, it shows a variable rate of malignant cells detection. Thus, we did this study to compare the positivity of LBC with CC in eastern Indian population.

Material and methods: A total of 150 patients who underwent a transurethral resection of bladder tumor (June 2017 to December 2018) were enrolled. Pre-operative CC and LBC were processed from freshly voided urine samples. The malignant cells detection rate and influential factors were noted and compared.

Results: The detection of malignant cells by LBC was higher compared to CC (37.3% vs. 25.3%; p<0.0001). Among 59 high-grade tumors, 59% and 86% slides were positive for CC and LBC, respectively (p<0.0001). Even in the background of hematuria, LBC showed a better detection (43.33% by LBC vs. 23.66% by CC; p<0.0001).

Conclusion: The present study concludes that LBC offers a better detection of malignant cells in the urine of patients with bladder tumor as compared to CC. The detection of malignant cells by LBC is even better in the background of hematuria.

Keywords: Bladder tumor; cytology; biomarkers; liquid-based cytology.

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Introduction

One of the most prevalent cancers worldwide is bladder cancer.[1] More than two-thirds of patients with bladder cancer present with the non-muscle-invasive variant and are managed by transurethral resection. However, even this non-muscle-invasive variant has a very high recurrence and progression. Due to its very high recurrence and progressive nature, it leads to significant mortality and morbidity.[2] Prompt detection decreases cancer-related mortality and morbidity.

Cystoscopy and urinary cytology have been widely employed and used for the detection, follow-up, and surveillance of bladder cancer. Cystoscopy is the most commonly used modality to diagnose and follow-up patients with bladder cancer. However, it is an operator-dependent, invasive, and painful procedure for patients. In addition, it may lead to false-negative interpretations.[3]

In the background of a high clinical burden with a costly and invasive cystoscopic procedure that further requires expertise, we need simpler, reproducible community-based tests such as urinary-based biomarkers. Conventional cytology (CC) is one of these biomarkers used for the detection of malignant cells in urine of patients affected by bladder cancer. It is readily available and community-based applicable test. It has a very high specificity (85%-100%),

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but it lacks good sensitivity. Sensitivity is likely influenced by external factors and the hostile environment of urine.[4]

Liquid-based cytology has been widely used for the detection of malignant cells in many diseases, such as cervical cancer, lung cancer, breast cancer, and bladder cancer.[5-7] An improved confounding cell-free background, decreased cell degeneration, and improved slide quality were noted in LBC.[8] Even after these positive effects, a variable detection of malignant cells in urine was observed.[7] This is the basis of our study, to know the positivity of LBC as compared to CC in eastern Indian population.

Material and methods

After the institutional ethical board approval from institutional ethical committee of Institute of Post-Graduate Medical Education & Research, Kolkata, India (IPGME&R/IEC/2018/261) and obtaining individual informed written consent, we conducted a prospective observational study including 150 patients who underwent transurethral resection of bladder tumor during the period between June 2017 and December 2018 in the Department of Urology, Institute of Post-Graduate Medical Education and Research, Kolkata, India. Patients were excluded on the basis of an active urinary tract infection, bladder stones, and associated upper-tract malignancy.

After the inclusion of patients, a detailed history was recorded, and 100 mL of freshly voided samples (morning samples were not taken) were collected preoperatively after the admission of patients and divided into two 50 mL samples. The samples were processed within 1 hour. One 50 mL samples were processed for CC and another for LBC.

The CC slide preparation was done by sediment obtained by double centrifugation of urinary samples. The slide was stained with Papanicolaou's method.

The LBC slide was made using the BD sure-path method. In a 50 mL test tube (provided by the manufacturer), the urine sample was added and mixed for 5 minutes to homogenize. Clots and gross contaminate were removed, followed by which samples were centrifuged for 10 mins at 300 g. Supernatant fluid was disposed of. BD cytorich red was added to the sediment, and the sample was vortexed and kept for 30 mins. Again, samples were centrifuged for 10 mins at 600 g. The resultant supernatant fluid was decanted, and the final sample was mixed again. If a moderate to large pellet was detected, two to five drops of representative samples were transferred to the 12 mL tube, and 10 mL of water was added and centrifuged for 5 mins for 600 g. Sediments were obtained after disposing supernatant fluid. Finally, 12 mL centrifuge tubes were loaded onto the BD Prep Stain slide processor, and the slide was made using an automated processor having non-gyn slide processor software.

Cytology reporting was done using the Paris system. For clinical and better understanding, we broadly stratified the report into positive and negative cytology for detection of malignant cells. Atypical or suspicious cytology was considered negative for malignancy in the present study.

After transurethral resection, histopathology reporting was also noted as the TNM staging. Grading was also recorded as per the World Health Organization's 2016 classification.

Statistical analysis

All data were collected, and the analysis was done with the IBM Statistical Package for the Social Sciences, Version 23.0 (IBM SPSS Corp.; Armonk, NY: USA). Pearson's chi-square test and Mc Nemar-Bowker tests were used to compare the positivity of CC and LBC.

Results

In the present study, 150 patients out of total admitted bladder tumor patients were included after exclusion of 39 patients (based on the exclusion criteria). The mean age of patients was 59.74±21.5 years, and most of the patients were older than 50 years of age and mostly were male and smokers (Table 1).

Ninety-four percent of study subjects presented with hematuria. Most of the tumors were non-muscle invasive and around two-thirds of tumors were low grade. Overall, 25.3% of CC was positive for malignant cells, and 37.3% of LBC slides were positive for malignant cells. This difference is statistically significant ($p<0.0001$) (Table 2).

Among 59 high-grade tumors, 59% of slides were positive for CC, while 86% were positive for LBC. The McNemar test showed a statistically significant difference ($p<0.0001$). But in low-grade tumors, a statistically insignificant difference of positivity (LBC 5.4% vs. 3.2%; $p=0.625$) was observed (Table 2).

Forty percent of patients had ongoing hematuria during urine collection, and 43.33% vs 23.66% of samples were positive for malignant cells in LBC and CC, respectively, in the background of hematuria. A greater positivity in LBC as compared to CC was statistically significant ($p<0.0001$) (Table 2).

Discussion

As bladder cancer leads to significant mortality and morbidity, early diagnosis and management decreases the burden and disease-specific mortality. Currently, cystoscopy, cytology, and upper-tract imaging are employed for diagnosis and follow-up in patients with bladder cancer.

Table 1. Clinical features, stage, and grading of tumor

Variables	Frequency	%
Age (years)	n=150 {mean age 59.74±21.5}	
<41 years	7	4.7
41-50	17	11.3
51-60	57	38.0
61-70	51	34.0
>71 years	18	12.0
Sex	n=150	
Male	126	84
Female	24	16
Family history	n=150	
Present	17	11.3
Absent	133	88.7
Smoking status	n=50	
Smoker	119 (112 male & 7 female)	79.3
Non-smoker	31 (14 male & 17 female)	20.7
Mode of presentation	n=150	
Hematuria	141	94
Others	9	6
Incidental	2	
Dysuria	5	
LUTS	2	
Ongoing hematuria during specimen collection	n=150	
Present	60	40
absent	90	60
Tumor stage	n=150	
Benign	2	1.3
PUNLMP	2	1.3
Ta	49	32.7
T1	82	54.7
T2	14	9.3
CIS	1	0.7
Grade of tumor	n=150	
Benign	2	1.3
PUNLMP	2	1.3
Low grade	87	58
High Grade	59	39.3

LUTS: lower urinary tract symptoms; PUNLMP: papillary urothelial neoplasia of low malignant potential; CIS: carcinoma in situ

Table 2. Cytology correlation

Variables	Frequency	Comments
Urinary malignant cell detection	n=150	
CC	38 (25.3%)	p<0.0001
LBC	56 (37.3%)	
Positive for malignant cell in low-grade tumor*	n=91	
CC	3 (3.2%)	p=0.625
LBC	5 (5.4%)	
Positive for malignant cell in high-grade tumor	n=59	
CC	35 (59.32%)	p<0.0001
LBC	51 (86.44%)	
Positive for malignant cell in ongoing hematuria during specimen collection	n=60	
CC	13 (21.66%)	p<0.0001
LBC	26 (43.33%)	

*low grade includes benign, PUNLMP and low-grade bladder neoplasm. LBC: liquid based cytology; CC: conventional cytology

As cystoscopy is an operator-dependent and painful procedure, it may lead to false-negative results and discomfort.[3] As a significant number of patients are suffering from bladder cancer, a simple, cost-effective, and reproducible investigation may be helpful for both the patient and the clinician. One of them is urinary-based CC.

One of the downsides of CC is a low detection rate of bladder cancer (approximately 20%-50%).[7] In our study, we established a similar result of a 25.3% positivity with CC (Table 2). Low detection is more pronounced in low-grade tumors due to less exfoliation (good cohesive nature of low-grade tumor) and a similar cytomorphology (to normal urothelial cell).[8] In our study, 3 out of 91 low-grade tumors showed positivity for malignant cytology (Table 2).

Another possible reason for a low detection of malignant cells by CC are background impurities such as mucus, blood, etc. (Figure 1), which hampers the detection of malignant cells. On the other hand, LBC is an automated and unique method of preparation of slide. Before an automated preparation of slide, urinary cells were fixed with a liquid-based preservative like cytorich red. It prepares the slide, which is thin layered and has a comparative haziness-free background (Figure 2).[8] Furthermore, nuclear outline and nuclear cytoplasmic ratio were well perceived with LBC (Figure 3 and 4).

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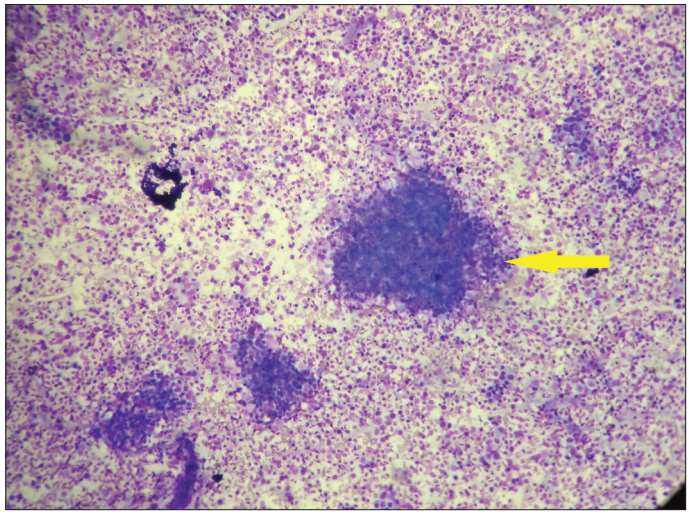

Figure 1. Conventional cytology positive for malignant cells (dense inflammatory background). The arrow shows a cluster of malignant cells

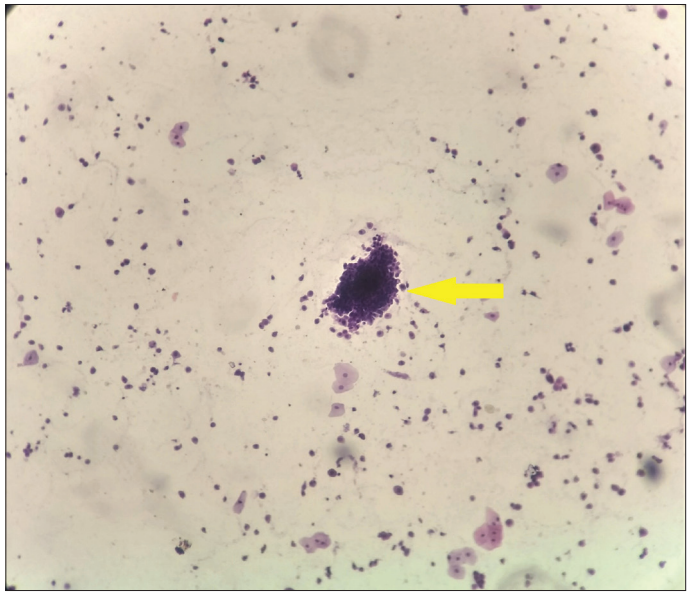

Figure 2. Liquid-based cytology positive for malignant cells (clear background). The arrow shows a cluster of malignant cells

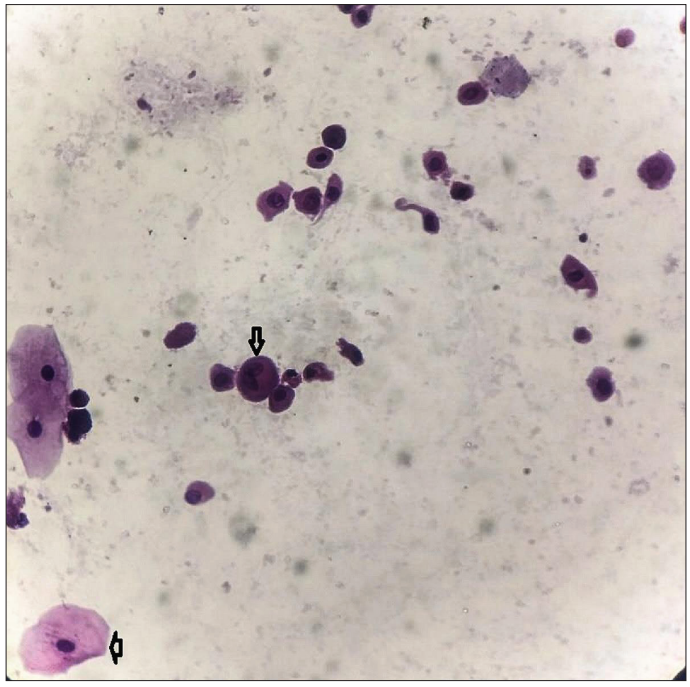

Figure 3. Liquid-based cytology. The vertical arrow shows binucleate malignant cells (clear nuclear outline), and the horizontal arrow shows normal urothelial cells

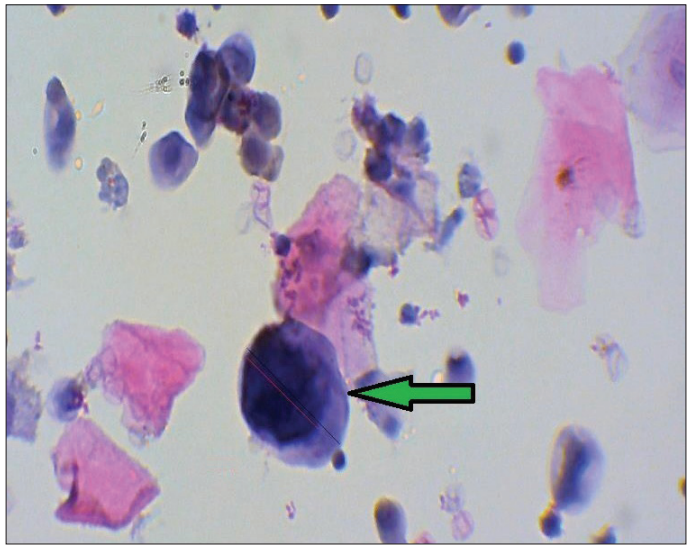

Figure 4. Liquid-based cytology. The arrow shows malignant cells with a high N/C ratio

Apparently haziness free background and thin-layered slide of LBC yields a wide range of sensitivity and detection of malignant cells in the urine of patients with bladder cancer. The lowest sensitivity of LBC was detected by Sullivan et al.[9]. They had studied a voided urine sample of 100 patients from November 2006 to March 2007, who were monitored for bladder cancer. They found that an overall sensitivity of liquid-based cytology for urothelial cell carcinoma was 21% (low grade 15% and high grade 27%) and the specificity was 97%, while Piaton et al.[10] found an 80% sensitivity and 91% specificity. They had collected 216 urine samples from patients who were referred for cystoscopy (n=92) or follow-up patients of conservatively managed bladder (n=117) and an upper-tract lesion (n=7). Based on previous studies, an overall sensitivity of LBC ranges from 21% to 80%.[7,10-19] In our study, we established a 37.3% slide positivity for malignant cells in LBC. This result is similar to previous studies (Table 2).

Now the question is whether LBC offers a better detection of malignant cells in urine of bladder cancer as compared to CC? Comparative studies between LBC and CC showed variable results. A study done by Son et al.[11] showed a better detection of malignant cells by LBC as compared to CC. It comprises of 713 voided specimens of urine collected in the Pathology Department of Chungbuk National University Hospital. They revealed 50% diagnostic sensitivity of LBC as compared to 37.5% of CC, while the study conducted by Sng et al.[12] had shown better result with CC. A total of 120 unfixed received urine samples were assessed by the preparation of slide by both LBC and CC. The sensitivity of conventional cytology (90.0%) was found to be higher compared to LBC (80%).

In the present study, an overall 37.3% detection of malignant cells in urine of bladder cancer by LBC was observed, which is higher when compared to 25.3% by CC. A greater percentage of positivity is statistically significant (Table 2). A better detection of malignant cells with LBC (as compared to CC) is supported in a study by Son et al.[11] in 2012. The overall detection of malignant cells by any means is <40% in the present study, and a probable reason for lower detection may be the inclusion of a greater number of patients having low-grade tumors (91 out of 150) and an analysis of only a single voided sample. In high-grade tumors (59 out of 150), we found a better detection of malignant cells by both methods (i.e., LBC and CC). Our study showed a statistically better positive detection by LBC in comparison to CC 86% vs 59%; p<0.0001).

As an additional finding in the current study, we also noticed that in the background of hematuria, the positivity of LBC was better than CC (43.33% vs 23.66%; p<0.0001) (Table 2). A plausible reason for a better detection of malignant cells in the background of hematuria is the slide preparation with a haziness-free background. This hypothesis is also supported by by Piaton et al.[10]

In conclusion, in the present study, we conclude that LBC offers a better detection of malignant cells in urine of patients with bladder tumor as compared to CC. Detection of malignant cells using LBC is even better with hematuria. Limitations to the present study are a small number of patients, single institution, and a non-blinded and non-randomized study.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Institute of post graduate medical education and research, Kolkata, India (IPGME&R/IEC/2018/261).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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References

1. Jemal A, Center M, DeSantis C, Ward E. Global Patterns of Cancer Incidence and Mortality Rates and Trends. Cancer Epidemiol Biomarkers Prev 2010;19:1893-907. [CrossRef]
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7-30. [CrossRef]
3. Tahoun NS, Abdel Maksoud AM, Mohamed DB. Evaluation of the Value of Combined Urine Cytology and Cystoscopy for Follow-up of Superficial Transitional Cell Carcinoma of the Urinary Bladder. J Egypt Natl Canc Inst 2010;22:105-11.
4. Raitanen M, Aine R, Rintala E, Kallio J, Rajala P, Juusela H, et al. Differences Between Local and Review Urinary Cytology in Diagnosis of Bladder Cancer. An Interobserver Multicenter Analysis. Eur Urol 2002;41:284-9. [CrossRef]
5. Gerhard R, Schmitt F. Liquid-Based Cytology in Fine-Needle Aspiration of Breast Lesions: A Review. Acta Cytologica 2014;58:533-42. [CrossRef]
6. Saraiya M. Liquid-Based Cytology vs Conventional Cytology in Detecting Cervical Cancer. JAMA 2010;303:1034. [CrossRef]
7. Luo Y, She D, Xiong H, Yang L, Fu S. Diagnostic Value of Liquid-Based Cytology in Urothelial Carcinoma Diagnosis: A Systematic Review and Meta-Analysis. PLoS One 2015;10:e0134940. [CrossRef]
8. Lu DY, Nassar A, Siddiqui MT. High-grade urothelial carcinoma: comparison of SurePath liquid-based processing with cytospin processing. Diagn Cytopathol 2009;37:16-20. [CrossRef]
9. Sullivan PS, Nooraie F, Sanchez H, Hirschowitz S, Levin M, Rao PN, et al. Comparison of ImmunoCyt, UroVysion, and urine cytology in detection of recurrent urothelial carcinoma: a "split-sample" study. Cancer 2009;117:167-73. [CrossRef]
10. Piaton E, Carre C, Advenier AS, Decaussin-Petrucci M, Mege-Lechevallier F, Lantier P, et al. p16 INK4a overexpression and p16/Ki-67 dual labeling versus conventional urinary cytology in the evaluation of urothelial carcinoma. Cancer Cytopathol 2014;122:211-20. [CrossRef]
11. Son SM, Koo JH, Choi SY, Lee HC, Lee YM, Song HG, et al. Evaluation of Urine Cytology in Urothelial Carcinoma Patients: A Comparison of CellprepPlus(R) Liquid-Based Cytology and Conventional Smear. Korean J Pathol 2012;46:68-74. [CrossRef]
12. Sng KK, Nga ME, Tan SY, Walker T. Analysis of urine cytology tests in 120 paired cases. Acta Cytol 2007;51:782-7. [CrossRef]

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DOI:10.5152/tud.2019.19040

13. Kim JY, Kim HJ. A Comparison Between ThinPrep Monolayer and Cytospin Cytology for the Detection of Bladder Cancer. Korean J Urol 2014;55:390-4. [CrossRef]
14. Li H, Tang R. A comparison of tct with traditional cytospin cytology in urinary cytology. Hebei Med J 2014;36:3079-81.
15. Dimashkieh H, Wolff DJ, Smith TM, Houser PM, Nietert PJ, Yang J. Evaluation of urovysion and cytology for bladder cancer detection: a study of 1835 paired urine samples with clinical and histologic correlation. Cancer Cytopathol 2013;121:591-7. [CrossRef]
16. Van Hemel BM, Haarsma JG, Ruitenbeek T, Groen HJ, Suurmeijer AJH. Application of the ThinPrep imaging system in urine cytology: a prospective study. Cancer Cytopathol 2013;121:410-4. [CrossRef]
17. Shen Z, Shen Z, Zhang M. Diagnosis of urothelial carcinoma of bladder by test paper of urinary fibronectin. J Contemp Urol Reprod Oncol 2013;5:274-78.
18. Li HX, Wang MR, Zhao H, Cao J, Li CL, Pan QJ. Comparison of fluorescence in situ hybridization, NMP22 bladderchek, and urinary liquid-based cytology in the detection of bladder urothelial carcinoma. Diagn Cytopathol 2013;41:852-7. [CrossRef]
19. Ye J, Zhu Y, Zhou X, Wang Q, Liu S. Diagnostic value of combination with lbc and molecular markers in bladder cancer detection. Shandong Med J 2011;51:55-6.