Original Research Article

A Hospital Based Comparative Assessment of the Efficacy of Direct Smear versus Liquid-Based Cytology in the Diagnostic Evaluation of Bladder Lesions

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Abstract

Aim: The purpose of this study was to evaluate urine cytology, detect bladder lesions with two different methods (i.e., direct smears and LBC), and determine the sensitivity and specificity of these methods.

Methods: This Observational study was conducted for a period of 1 year at Department of Pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India. A total of 200 samples were taken from patients with suspected bladder cancer and processed for direct smear and LBC. In both methods, findings were reported according to the Paris System. Then, patients underwent cystoscopy and biopsy. Next, the accuracy of cytology methods was evaluated according to biopsy reports. The sensitivity and specificity of these methods were also calculated.

Results: NUAM in LBC and DSC were 80% and 79% respectively. In this study, the largest difference was reported in patients with a diagnosis of AUC in the LBC method. In these 16 cases, 16 cases were reported as negative in DSC. Kappa statistics revealed a significant agreement between LBC and DSC in negative biopsy cases (P<0.000), Kappa value=0.650. In the frequency of biopsy findings, 80% were negative. For DSC methods, credit indices included sensitivity (61.9%), specificity (98%), positive predictive value (89.1%), and negative predictive value (91.5%). For LBC methods, credit indices included sensitivity (84.6%), specificity (99%), positive predictive value (94%), and negative predictive value (96%).

Conclusion: This study showed that LBC has higher sensitivity and specificity than the direct smear.

Keywords: Bladder Cancer, Direct Smear, Liquid-Based Cytology, Urine Cytology.

Introduction

Bladder cancer is one of the most common cancers of the urinary system. [1] Bladder urothelial cell carcinoma is a heterogeneous group of tumors with different malignant potentials. [2,3] Approximately 80% of bladder cancers are low-grade superficial tumors [4]. Routinely, cystoscopy and cytology are used to diagnose and follow up superficial bladder tumors [5]. Cystoscopy is the most efficient method available to detect primary or recurrent bladder cancer [6].Therefore, it is important to use urine cytology as a noninvasive complementary method [7,8] Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is widely applied to the histological diagnosis of bladder cancer. [9] The diagnostic performance of this technique varies depending on several factors, including tumor size, tumor location, and tumor characteristics. [10]

Specimens collected by EUS-FNA have traditionally been analyzed using smear cytology (SC), which has become the standard method of cytological diagnosis. It has a 95% sensitivity and nearly 100% specificity in detecting high-grade urothelial malignancies. [11] However, it is a low sensitive method for detecting low-grade malignant urothelial tumors (the most common urothelial carcinoma) [12] and SC shows some disadvantages, such as cell crowding and blood contamination. One of the recent methods is Liquid-based cytology (LBC) is a thin-layer slide preparation procedure that was developed to overcome the cell crowding and contamination issues associated with SC. [13] The diagnostic value of cervical cytology uterine cervical cancer using LBC is now established worldwide and

breast cancer, thyroid cancer and lymphoma have been assessed using LBC. Compared to direct smear cytology (DSC), LBC has lower background elements (such as cellular debris, inflammatory cells, and blood cells), provides better cell preservation, and has a higher satisfaction rate. [14,15] In the LBC method, after adding the fixative solution, all extracted cells were maintained; therefore, there are more cells for cytological examination.

The purpose of this study was to evaluate urine cytology, detect bladder lesions with two different methods (i.e., direct smears and LBC), and determine the sensitivity and specificity of these methods.

Materials & Methods

This Observational study was conducted for a period of 1 year at department of Pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India .A total of 200 patients suspected of having bladder carcinoma and their urine specimens were examined by two cytology methods (direct smear and LBC); also, their sensitivity and specificity were compared. All urine samples were collected at midday to prevent false-positive results (morning samples were not taken). The patients were advised to drink a few glasses of water 1-2 h before sampling. All samples were freshly voided urine. Urine samples were taken in our laboratory or immediately transferred to our laboratory; instrumented urine specimens were excluded. The gross nature of samples (such as color, volume, and clarity) was recorded. In our laboratory, each specimen was divided into two halves for further processing. One-half of the voided sample was prepared for the direct smear method and another half for the LBC method. To prevent cell damage, 10% formalin or 50% alcohol was equally added to the sample size immediately.

In the direct smear method, slide preparation was done by sediment obtained from centrifugation of urinary samples (1000 rpm for 30 min). The slide was stained with Papanicolaou and hematoxylin and eosin (H&E) staining methods.

In LBC, samples were centrifuged at 1500 rpm, and the precipitates were transferred to 30 mL of a Cytolytic solution (Sina Dej Sahand Co, Tabriz, Iran). After another round of centrifugation, two or three drops of precipitates were transferred to the preservative solution (Sina Dej Sahand Co, Tabriz,

Iran). The vial and slide were placed in a Thin Prep processor (SHANDON, CYTOSPIN3, UK).

The preparation steps are as follows:

1) dispersion: filter rotates within the sample vial, creating currents in the fluid that are strong enough to separate debris and disperse mucus but gentle enough to have no adverse effect on cell appearance;

2) cell collection: a gentle vacuum is created within the Thin Prep Filter, which collects cells on the exterior surface of the membrane;

3) cell transfer: after collecting the cells on the membrane, the Thin Prep Filter is inverted and gently pressed against the Thin Prep Microscope Slide.

Natural attraction and slight positive air pressure cause the cells to adhere to the Thin Prep Microscope Slide, resulting in an even distribution of cells in a defined circular area. Then, ethanol 95% is used for fixation; and

4) staining: Papanicolaou and H&E staining methods were performed manually.

In both methods, the findings were reported according to the Paris System. Thereafter, all patients underwent cystoscopy and biopsy, and the precision of both cytology methods was compared according to biopsy results.

This study included all patients with suspicious symptoms and signs of bladder carcinoma or recurrent disease and all those patients with a history of bladder carcinoma referred for follow-up studies. We excluded patients with incomplete cytology, cystoscopy, and biopsy procedures from the study.

Statistical Analysis

Data collected from clinical and laboratory observations were analyzed using SPSS 21 (SPSS Inc., Chicago, Ill., USA). The clinical and laboratory characteristics of patients were described by descriptive statistical methods, and Student t-test, chi-square, McNemar, and Kappa tests were used. The sensitivity, specificity, positive, and negative predictive values of direct cytology and liquid-based methods were also calculated and compared based on final biopsy results.

Results

Table 1: Frequency of liquid-base and direct smear cytology findings

Finding	LBC	DSC
NUAM	160 (80%)	158 (79%)
AUC	16 (8%)	16 (8%)
LGUN	4 (2%)	4 (2%)
SHGUC	8 (4%)	12 (6%)
HGUC	12 (6%)	10 (5%)

NUAM in LBC and DSC were 80% and 79% respectively. In this study, the largest difference was reported in patients with a diagnosis of AUC in the LBC method. In these 16 cases, 16 cases were reported as negative in DSC. Kappa statistics revealed a significant agreement between LBC and DSC in negative biopsy cases (P<0.000), Kappa value=0.650.

Table 2: Frequency of biopsy findings

Findings	Frequency	Percent
Negative	160	80%
Dysplasia	4	2%
CIS	4	2%
LGTCC	14	7%
HGTCC	18	9%
Total	200	100

In the frequency of biopsy findings, 80% were negative.

Table 3: Direct smear cytology and liquid-based cytology statistics

	DSC	LBC
Sensitivity	61.9%	84.6%
Specificity	98%	99%
Positive Predictive Value	89.1%	94%
Negative Predictive Value	91.5%	96%

For DSC methods, credit indices included sensitivity (61.9%), specificity (98%), positive predictive value (89.1%), and negative predictive value (91.5%). For LBC methods, credit indices included sensitivity (84.6%), specificity (99%), positive predictive value (94%), and negative predictive value (96%).

Discussion

Cystoscopy is the most efficient method available to detect primary or recurrent bladder cancer. [16] Yet cystoscopy is an invasive procedure and causes some discomfort in patients, and it might be ineffective in diagnosing in situ or superficial tumors. [16-18] Therefore, it is important to use urine cytology as a noninvasive complementary method. [19,20] Urine cytology is an important noninvasive diagnostic method for urinary tract cancers, especially carcinomas. [20,21] It has a 95% sensitivity and nearly 100% specificity in detecting high-grade urothelial malignancies. [22] However, it is a low-sensitive method for detecting low-grade malignant urothelial tumors (the most common urothelial carcinoma). [23,24] It is useful for follow-up in treated patients and to evaluate the residual of malignant bladder tumors after surgery. [25] One of the recent methods is liquid-based cytology (LBC), which was used in cervical cytology for the first time. [26]

Only a few studies have compared these two cytology techniques in urine specimens. In the analysis of 236 urine specimens conducted by Lee et al., it was shown that the use of a Thin Prep-based liquid-based preparation method was useful to improve the quality of the slides and reduce the duration of the test, but the sensitivity, accuracy, and predictive value were not changed. [27] In another study, Koh et al. pointed out that the use of Cell Prep Plus LBC for body liquids had a higher sensitivity and higher negative predictive value. The quality of the slides was better than DSC. [28] Therefore, it is a useful diagnostic method in body fluid screening.

Liquid-based cytology (LBC) is a thin-layer slide preparation procedure that was developed to overcome the cell crowding and contamination issues associated with SC. [29] The diagnostic value of cervical cytology uterine cervical cancer using LBC is now established worldwide [30] and breast cancer, [31] thyroid cancer, [32] and lymphoma [33] have been assessed using LBC. However, comparisons of diagnostic accuracy between SC and LBC for various diseases, including pancreatic lesions, have yielded controversial findings. [34,35] For DSC methods, credit indices included sensitivity (61.9%), specificity (98%), positive predictive value (89.1%), and negative predictive value (91.5%). For LBC methods, credit indices included sensitivity (84.6%), specificity (99%), positive predictive value (94%), and negative predictive value (96%). However, in Fakhrjoo et al.'s study, the sensitivity and specificity of DSC in the diagnosis of bladder tumors of 900 patients were 73% and 99%, respectively. [36] In 2009, Lu et al. compared Cell Prep Plus LBC with the conventional smear in 713 patients. The diagnostic sensitivity for CellPrepPlus was 50% and higher than 37.5% for the conventional smear. The specificity of both preparations was 100%. [24] The

results of previous comparisons of diagnostic performance between SC and LBC for various diseases are controversial. Siebers et al[34] reported that the performance of LBC in terms of relative sensitivity and PPV for detecting cervical cancer precursors was not any better than that of conventional SC. In contrast, Son et al[35] found that LBC could reveal more cellularity with a cleaner background and better cytomorphological features and deliver markedly higher diagnostic sensitivity than SC.

Conclusion

This study showed higher sensitivity and higher specificity of Thin prep liquid-based cytology than direct smear cytology, especially in the diagnosis of Urothelial tumors with low-grade malignancy. LBC method can reduce AUC cases which is the waste-basket for pathologists. Furthermore, it lowers the unsatisfactory cases in DSC. In conclusion, although the LBC method costs more, its several advantages over the DSC method make it an appropriate alternate method to evaluate urinary samples.

References

1. Compérat EM, Burger M, Gontero P, Mostafid AH, Palou J, Rouprêt M, van Rhijn BW, Shariat SF, Sylvester RJ, Zigeuner R, Babjuk M. Grading of urothelial carcinoma and the new "World Health Organisation classification of tumours of the urinary system and male genital organs 2016". European urology focus. 2019 May 1;5(3):457-66.
2. Inamura K. Bladder cancer: new insights into its molecular pathology. Cancers. 2018 Apr 1; 10(4):100.
3. Pasin E, Josephson DY, Mitra AP, Cote RJ, Stein JP. Superficial bladder cancer: an update on etiology, molecular development, classification, and natural history. Reviews in urology. 2008;10(1):31.
4. Zieger K, Wolf H, Olsen PR, Højgaard K. Long-term follow-up of noninvasive bladder tumours (stage Ta): recurrence and progression. BJU international. 2000 May; 85 (7):824-8.
5. Anastasiadis A, de Reijke TM. Best practice in the treatment of nonmuscle invasive bladder cancer. Ther Adv Urol. 2012;4(1):13-32
6. Zhu CZ, Ting HN, Ng KH, Ong TA. A review on the accuracy of bladder cancer detection methods. Journal of cancer. 2019;10(17):4038.
7. Ozen H, Hall MC. Bladder cancer. Curr Opin Oncol. 2000;12(3):255-9.
8. Truta A, Popon TA, Saraci G, Ghervan L, Pop IV. Novel non invasive diagnostic strategies in bladder cancer. Clujul Medical. 2016;89 (2): 187.
9. Agarwal B, Krishna NB, Labundy JL, Safdar R, Akduman EI. EUS and/or EUS-guided FNA in patients with CT and/or magnetic resonance imaging findings of enlarged pancreatic head or dilated pancreatic duct with or without a dilated common bile duct. Gastrointestinal endoscopy. 2008 Aug 1;68(2):237-42.
10. Haba S, Yamao K, Bhatia V, Mizuno N, Hara K, Hijioka S, Imaoka H, Niwa Y, Tajika M, Kondo S, Tanaka T. Diagnostic ability and factors affecting accuracy of endoscopic ultrasound-guided fine needle aspiration for pancreatic solid lesions: Japanese large single center experience. Journal of gastroenterology. 2013 Aug; 48:973-81.
11. Planz B, Jochims E, Deix T, Caspers HP, Jakse G, Boecking A. The role of urinary cytology for detection of bladder cancer. European Journal of Surgical Oncology (EJSO). 2005 Apr 1;31(3):304-8.
12. Lu DY, Nassar A, Siddiqui MT. High-grade urothelial carcinoma: Comparison of SurePath liquid-based processing with cytospin processing. Diagnostic Cytopathology. 2009 Jan;37(1):16-20.
13. Hutchinson ML, Zahniser DJ, Sherman ME, Herrero R, Alfaro M, Bratti MC, Hildesheim A, Lorincz AT, Greenberg MD, Morales J, Schiffman M. Utility of liquid-based cytology for cervical carcinoma screening: results of a population-based study conducted in a region of Costa Rica with a high incidence of cervical carcinoma. Cancer Cytopathology: Interdisciplinary International Journal of the American Cancer Society. 1999 Apr 25;87(2): 48-55.
14. Fakhrjou A, Madaen S. Specificity and Sensitivity of Urine Cytology for Screening Patients with Urothelial Tumors and Hematuria.
15. Kord S, Mokhtari M, Tahmasebi S. Comparison of liquid-based and conventional cytology in diagnosis of breast mass. Journal of cytology. 2019 Jan;36(1):22.
16. Zhu CZ, Ting HN, Ng KH, Ong TA. A review on the accuracy of bladder cancer detection methods. J Cancer. 2019;10(17):4038-44.
17. Wiener HG, Mian C, Haitel A, Pycha A, Schatzl G, Marberger M. Can urine bound diagnostic tests replace cystoscopy in the management of bladder cancer? J Urol. 1998; 159(6):1876-80.
18. Stamatiou K, Papadoliopoulos I, Dahanis S, Zafiropoulos G, Polizois K. The accuracy of ultrasonography in the diagnosis of superficial bladder tumors in patients presenting with hematuria. Ann Saudi Med. 2009;29(2):134-7.
19. Ozen H, Hall MC. Bladder cancer. Curr Opin Oncol. 2000;12(3):255-9.

20. Truta A, Popon TA, Saraci G, Ghervan L, Pop IV. Novel non invasive diagnostic strategies in bladder cancer. Clujul Med. 2016;89(2):187-92.

21. Hoda RS. Non-gynecologic cytology on liquid-based preparations: A morphologic review of facts and artifacts. Diagn Cytopathol. 2007;35(10):621-34.

22. Planz B, Jochims E, Deix T, Caspers HP, Jakse G, Boecking A. The role of urinary cytology for detection of bladder cancer. Eur J Surg Oncol. 2005;31(3):304-8.

23. Linder J. Recent advances in thin-layer cytology. Diagn Cytopathol. 1998;18(1):24-32.

24. Lu DY, Nassar A, Siddiqui MT. High-grade urothelial carcinoma: comparison of SurePath liquid-based processing with cytospin processing. Diagn Cytopathol. 2009;37(1):16-20.

25. V L. New techniques In: Kocjan WGG, editor. Diagnostic Cytopathology. 1. 3rd ed. london: Churchill Livingstone; 2010. p. 891-902.

26. Denton KJ. Liquid based cytology in cervical cancer screening. Bmj. 2007;335(7609):1-2.

27. Lee S, Park JH, Do SI, Kim YW, Lee JH, Chang SG, Park YK. Diagnostic value of urine cytology in 236 cases; a comparison of liquid-based preparation and conventional cytospin method. The Korean Journal of Cytopathology. 2007;18(2):119-25.

28. Koh JS, Cho SY, Ha HJ, Kim JS, Shin MS. Cytologic Evaluation of CellPrep(R) Liquid-based Cytology in Cervicovaginal, Body Fluid, and Urine Specimens: Comparison with ThinPrep(R). J Pathol Transl Med. 2007; 18 (1):29-35.

29. Hutchinson ML, Zahniser DJ, Sherman ME, Herrero R, Alfaro M, Bratti MC, Hildesheim A, Lorincz AT, Greenberg MD, Morales J, Schiffman M. Utility of liquid-based cytology for cervical carcinoma screening: results of a population-based study conducted in a region of Costa Rica with a high incidence of cervical carcinoma. Cancer Cytopathology: Interdisciplinary International Journal of the American Cancer Society. 1999 Apr 25;87(2): 48-55.

30. Cheung AN, Szeto EF, Leung BS, Khoo US, Ng AW. Liquid-based cytology and conventional cervical smears: a comparison study in an Asian screening population. Cancer Cytopathology: Interdisciplinary International Journal of the American Cancer Society. 2003 Dec 25;99(6):331-5.

31. Yamashiro K, Taira K, Nakajima M, Okuyama D, Azuma M, Takeda H, Suzuki H, Jotoku H, Watanabe K, Takahashi M, Taguchi K. Tissue rinse liquid-based cytology: a feasible tool for the intraoperative pathological evaluation of sentinel lymph nodes in breast cancer patients. Cytopathology. 2012 Aug;23(4):263-9.

32. Lee SH, Jung CK, Bae JS, Jung SL, Choi YJ, Kang CS. Liquid-based cytology improves preoperative diagnostic accuracy of the tall cell variant of papillary thyroid carcinoma. Diagnostic cytopathology. 2014 Jan;42(1):11-7.

33. Rossi ED, Larghi A, Verna EC, Martini M, Galasso D, Carnuccio A, Larocca LM, Costamagna G, Fadda G. Endoscopic ultrasound-guided fine-needle aspiration with liquid-based cytologic preparation in the diagnosis of primary pancreatic lymphoma. Pancreas. 2010 Nov 1;39(8):1299-302.

34. Siebers AG, Klinkhamer PJ, Grefte JM, Massuger LF, Vedder JE, Beijers-Broos A, Bulten J, Arbyn M. Comparison of liquid-based cytology with conventional cytology for detection of cervical cancer precursors: a randomized controlled trial. Jama. 2009 Oct 28;302(16):1757-64.

35. Biscotti CV, Hollow JA, Toddy SM, Easley KA. ThinPrep versus conventional smear cytologic preparations in the analysis of thyroid fine-needle aspiration specimens. American journal of clinical pathology. 1995 Aug 1;104(2):150-3.

36. Fakhrjou a, madaen sk. Specificity and sensitivity of urine cytology for screening patients with urothelial tumors and hematuria. Medical journal of tabriz university of medical sciences. 2003;37(57).