Section: Pathology

Original Research Article

COMPARATIVE STUDY ON DIFFERENT PREPARATION METHODS IN URINE CYTOLOGICAL ANALYSIS AS POTENT SCREENING TOOL FOR MALIGNANCIES AFFECTING THE URINARY TRACT

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Abstract

Background: Cytology tests are clinically valuable and straightforward to perform, with minimal sample preparation and handling requirements. It is also reliable because of its high sensitivity and specificity. This study aimed to compare EziPREP™, cytology smears, and other methods of obtaining thin-layer cytologic preparations (cytocentrifugation, direct smearing, and Nucleopore filtration) in urine cytopathology. **Materials and Methods:** This comparative cross-sectional study included 50 urine samples for cytological evaluation. The patients were assessed for urinary symptoms and medication histories. Urine samples were processed using EziPREP™, Cytospin, direct smearing, and Nucleopore filtration methods. The sensitivity, specificity, PPV, and NPV were calculated for each technique. **Results:** Among the 50 urine samples analysed, 58% were from female patients, with the majority aged <55 years. Cytological analysis revealed that Cytospin and EziPREP™ showed equal diagnostic performance, each achieving a sensitivity of 100%, specificity of 97.8%, PPV of 75%, and NPV of 100%. The Nucleopore filtration method also showed 100% sensitivity and high diagnostic accuracy; however, two smears were deemed unsatisfactory and were excluded from the final analysis. **Conclusion:** LBC EziPREPTM exhibited good sensitivity and specificity, and Cytospin was as reliable as LBC EziPREPTM in detecting HPV. Although the filter method had high sensitivity and other diagnostic values, the filter method smears showed some unsatisfactory results in cytology.

INTRODUCTION

Bladder urothelial cell carcinoma comprises diverse tumours with varying malignant potential and natural histories. Approximately 80% of bladder cancers present as superficial, low- or intermediate-grade tumours. While these tumours can be effectively resected via transurethral resection, they have a high recurrence rate (60–85%), with the highest incidence occurring within the first year.[1,2] Despite this risk, the 5-year survival rate remains favourable, ranging from 80% to 90%. Given these characteristics, the primary goal in managing superficial bladder cancer is to reduce and delay recurrences while preventing progression to invasive disease, necessitating long-term monitoring.[3]

Cystoscopy and urine cytology are the most commonly used diagnostic and surveillance tools for superficial bladder tumours. Cystoscopy remains the gold standard for detecting primary and recurrent urothelial carcinoma. However, it is an invasive procedure associated with patient discomfort and has limitations in detecting flat lesions, such as carcinoma in situ.[4] There is a growing need for a reliable non-invasive test to complement standard diagnostic methods. Urine cytology is a valuable alternative, offering high sensitivity and specificity with minimal sample preparation and handling.[5,6] Traditionally, urine cytology has been performed using conventional methods such as cytocentrifugation, filtration, and direct smearing. However, these techniques have inherent limitations, including low cellular yield, overlapping cells, suboptimal cell preservation, and background artefacts caused by inflammatory cells and cellular debris.[7] To overcome these challenges, liquid-based

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cytology (LBC) has emerged as a superior alternative, significantly improving diagnostic accuracy and efficiency of the procedure. Initially developed for cervical cytology, LBC has been adapted for urine cytopathology, providing improved cellular preservation, reduced background artefacts, and uniform cell distribution for microscopic evaluation of urine samples. In this technique, urine samples are first centrifuged to concentrate the cellular material. The precipitate is then suspended in a cytolytic solution and processed using an automated ThinPrep® system, ensuring optimal cell transfer and staining for accurate cytological assessment.[8-10]

Urine cytology remains a valuable non-invasive tool for detecting urinary tract malignancies, particularly high-grade urothelial carcinomas, with a reported sensitivity of 95% and near-perfect specificity. However, its effectiveness in detecting low-grade urothelial tumours, the most common subtype, remains limited. LBC, with its improved processing techniques, addresses some of these challenges by reducing background elements (e.g. inflammatory cells and blood cells), enhancing cell preservation, and providing a higher diagnostic satisfaction rate.[11]

By utilising fixative solutions, LBC ensures better cell retention, ultimately improving cytological evaluation. Thus, LBC represents a significant advancement in urine cytology, presenting a more reliable and efficient diagnostic approach for the surveillance of urothelial carcinoma.

Aim

This study aimed to compare EziPREP™, cytology smears, and other methods of obtaining thin-layer cytologic preparations (cytocentrifugation, direct smearing, and Nucleopore filtration) in urine cytopathology.

MATERIALS AND METHODS

This comparative cross-sectional observational study included 50 urine samples received for cytological evaluation at the Department of Pathology, Tagore Medical College and Hospital, Chennai, from June 2022 to December 2022. The study was conducted after approval from the institutional ethics committee, and written consent was obtained from the patient before the start of the study.

Inclusion and exclusion criteria

Urine samples were included if adequately labelled and submitted for complete urine analysis, obtained from individuals aged >35 years of both sexes. Only freshly voided, midstream, early morning urine samples collected within two hours were considered. Additionally, samples must be refrigerated at 1–3°C within eight hours of collection.

Samples were excluded if they were improperly labelled or collected, if clinical data were unavailable, if the patient was receiving treatment for a urinary tract infection or chemoprophylaxis, or if the patient had a confirmed diagnosis of malignancy.

Methods

The sample size was calculated using the formula n=4pq/d2, where p (prevalence rate) was 7%, q=100−pq = 100 − p, and d (margin of error) was 5%. This resulted in a sample size of 46, which was adjusted to 50 after accounting for a 10% nonresponse rate.[12]

Patients underwent a comprehensive evaluation, including an assessment of urinary symptoms and medication history. Relevant clinical data, including cystoscopy, ultrasonography (USG), computed tomography (CT), and magnetic resonance imaging (MRI) findings, were retrieved from the case records. Early morning midstream urine samples were collected in sterile containers, with fresh samples processed within two hours and refrigerated samples (1–3°C) processed within eight hours.

For EziPREP™ processing, urine samples were centrifuged using the KJLC-I® centrifuge at 500 g for 2 min, and slides were prepared using the non-GYN PreservCyt vial for 2 min. In the filtration method, a nucleopore polycarbonate filter (47 mm diameter, 8 µm pores) was rinsed with 95% ethanol and smeared using a sterile spatula. The conventional direct method used the REMI™ R 8C centrifuge at 800 rpm, and smears were prepared from the obtained supernatant. Conventional cytocentrifugation utilises the cytospin technique at 600 g to preserve cellular morphology. The slides were stained with Papanicolaou (Pap), haematoxylin-eosin (HE), and Leishman stains for morphological analysis.

Statistical Analysis

The data obtained were entered into Microsoft Excel and analysed using SPSS version 25. The results are presented as proportions. The diagnostic value was assessed by calculating the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The McNemar test was used to determine significance, with $p<0.05$ considered significant.

RESULTS

Most patients were aged <55 years, 58% were female, and 92% were cytology-negative on cytocentrifugation. Cytocentrifugation showed that the majority of patients (92%) were negative for cytological abnormalities, abnormal clusters were identified in 4%, and atypical cells and benign atypia were observed in 2% of patients each. [Table 1]

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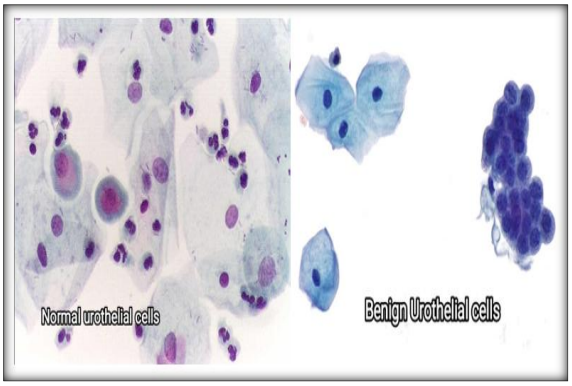

Figure 1: Normal and benign urothelial cells

A comparison of the cytological method results showed that 4% were positive according to Cytospin and EziPREP™, 4% were suspicious according to EziPREP™ and Cytospin, and 6% had clusters in the filter method. [Table 2]

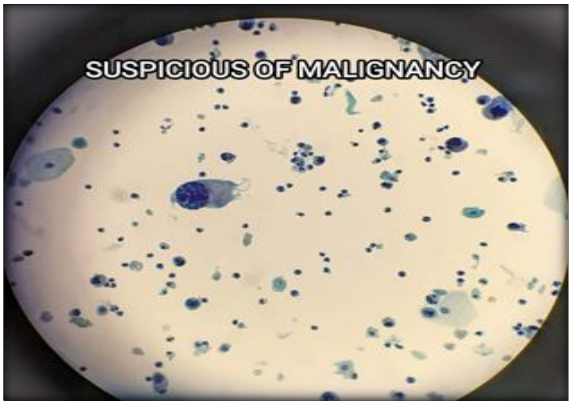

Figure 2: Suspicious of malignant cells

The cytospin sensitivity, specificity, PPV, and NPV were 100%, 97.8%, 75%, and 100%, respectively. Similarly, the sensitivity, specificity, PPV, and NPV of EziPREPTM were 100%, 97.8%, 75%, and 100%, respectively. The filter method had a sensitivity, specificity, PPV, and NPV of 100%. However, two smears obtained using the filter method were deemed unsatisfactory and excluded from the analysis. [Tables 3 and 4]

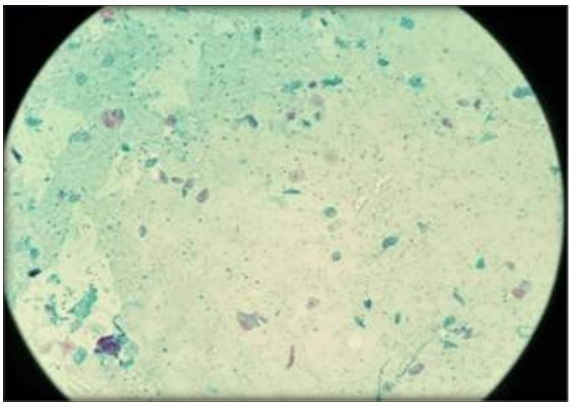

Figure 3: Malignant cells

The image shows benign, suspicious, and malignant urothelial cells every day, as per EziPREPTM. [Figure 3]

Table 1: Patient demographics and cytological diagnosis

		N (%)
Age (years)	35-44	25 (50%)
	45-54	14 (28%)
	55-65	8 (16%)
	65-74	2 (4%)
	>74	1 (2%)
Gender	Male	21 (42%)
	Female	29 (58%)
Cytological diagnosis as per Cytocentrifugation	Abnormal clusters	2 (4%)
	Atypical cells	1 (2%)
	Benign atypia	1 (2%)
	Negative	46 (92%)

Table 2: Comparison of cytological methods

Methods		N (%)
Cytospin	Suspicious	2 (4%)
	Negative	46 (92%)
Filter	Cluster	3 (6%)
	Negative	45 (90%)
EziPREP™	Positive	2 (4%)
	Suspicious	2 (4%)
	Negative	46 (92%)

Table 3: Table 3: PPV and NPV of cytological methods

		PPV	NPV
Cytospin	Positive/Suspicious	3	1
	Negative	0	46
EziPREP™	Positive/suspicious	3	1

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	Negative	0	46
Filter method	Positive/suspicious	3	0
	Negative	0	45

Table 4: Sensitivity and sensitivity of cytological methods

	Sensitivity	Specificity	PPV	NPV
Cytospin	100%	97.80%	75%	100%
EziPREP™	100%	97.80%	75%	100%
Filter method	100%	100%	100%	100%

DISCUSSION

Cytology has been used extensively for screening malignant neoplasms since 1939. However, the difficulties associated with CS, such as thick smears, overlapping cellular areas, low cellularity, obscuring inflammatory cells, blood, and air-drying artefacts, have made diagnosis difficult and resulted in low diagnostic sensitivity. As a result, LBC was introduced as a replacement for the traditional method, which has grown in popularity over the last two decades.[13]

Traditional urine cytology methods include cytocentrifugation, Millipore filtration, and direct smearing. Cytocentrifuge processing may result in a low cell yield and non-uniform, thickly smeared cells with poor cellular preservation. Unlike urine, conventional gynaecologic and non-gynaecologic preparations do not have problems with low cellularity. However, their high cellular content necessitates longer screening time owing to the increased number of slides per specimen and non-uniform screening area. Instead of being smeared, the cells are rinsed into a liquid collection medium and processed automatically with LBC. LBC has a higher cell yield than CS and reduces obscuring elements, solving one of the significant problems with low cellularity in urine cytology.[14] Lee et al. and Koo et al. found that using liquid-based preparations improved the quality of the slides and reduced the duration of a microscopic examination, but did not show enhanced sensitivity, accuracy, and predictive values, which contrasts with our study report.[14,15]

Our study demonstrated that both Cytospin and EziPREP ™ achieved 100% sensitivity, 97.8% specificity, 75% PPV, and 100% NPV, whereas the filter method reported 100% across all parameters but produced suboptimal cytological smears. These findings suggest that LBC and Cytospin are more effective for evaluating urothelial cancer. Additionally, Joo et al. found that monolayer preparation (MP) outperformed conventional preparation (CP) in terms of sensitivity (92.7% vs. 90.2%), specificity (87.2% vs. 66.2%), and likelihood ratios, with a notable reduction in atypical squamous cell classifications. These results further highlight the advantages of LBC over conventional methods.[16]

In the study by Kalantari et al., the sensitivity and specificity of Direct Smear Cytology (DSC) were reported as 60.7% and 98%, respectively, while for LBC, they were 85.7% and 99%.8 Similarly, Fakhrjou et al., in a study involving 900 patients, reported that the sensitivity and specificity of DSC for diagnosing bladder tumours were 73% and 99%, respectively.[17] Kapoor et al. found that LBC was more effective than Conventional Cytology (CC) in detecting malignant cells, with detection rates of 37.3% for LBC compared to 25.3% for CC.[18] Additionally, Lu et al. compared LBC with the conventional smear and found that LBC had a higher diagnostic sensitivity (50%) than the traditional smear (37.5%), while both techniques demonstrated high specificity.[10]

Son et al. reported that, compared with CS processing, there was no inadequacy due to low cellularity or thick preparation because malignant urothelial cells were distributed in a thin layer with less overlapping on LBC. LBC can also help identify malignant cells. LBC had a less cohesive architectural pattern than CS, with ball-shaped loose clusters of cells or scattered, single cells. The less cohesive pattern of LBC is thought to be influenced by cell filtration during its processing. This did not affect the accuracy of the diagnosis. This study also found that LBC cells were generally larger than those found in CS. Both LBC and CS exhibit nuclear features resembling those of urothelial carcinoma. LBC, however, shows more enlarged and translucent nuclei, making it easier to identify nuclear details such as nucleoli or chromatin changes.[7]

CONCLUSION

EziPREP™ LBC is notable for its increased cellularity, clean background, and increased preservation of cytomorphologic features, providing a more definitive diagnosis and potentially replacing conventional methods. Our study shows that LBC EziPREP™ has good sensitivity and specificity and that cytospin is as reliable as LBC EziPREP™. Although the filter method had high sensitivity and other diagnostic values, the filter method smears showed some unsatisfactory results in cytology. Therefore, EziPREP™ LBC and cytospin can be considered better and more highly predictive methods for assessing urolithiasis-related cancer.

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