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Usefulness of liquid-based preparation in urine cytology

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Objective: ThinPrep (TP), a liquid-based cytological and non-invasive technique to confirm the diagnosis of bladder cancer, is reported to be a better screening test than the conventional cytospin method. This study compared the new MonoPrep2 (MP), a liquid-based cytological technique, with TP for diagnosing bladder cancer.

Materials and Methods: Between January 2003 and June 2004, urine samples from 284 patients were processed using the TP and MP methods. The cytological diagnosis and the determination of specimen quality were performed separately. The cytological diagnoses were classified into four categories: unsatisfactory, benign, borderline, and malignant. A subsequent biopsy was performed in 73 patients. The cytological diagnoses were compared with the biopsy results to evaluate the sensitivity and specificity of the two methods.

Results: Considering all the features examined, the overall specimen quality was comparable between the MP and TP techniques in the majority of cases. The rate of satisfactory specimens was 100% for TP and 98.6% for MP. The diagnostic capacity was similar between MP and TP. The overall sensitivities with MP and TP were 58.6 and 62.0%, respectively, and the specificities were 100 and 97.7%; the differences were not significant ($P > 0.05$).

Conclusions: MP and TP produced comparable results in diagnosing bladder cancer. As MP is less expensive than TP, we recommend MP as an alternative liquid-based cytology method for use in bladder cancer screening.

Key words: bladder tumor, liquid based cytology, urine cytology.

Introduction

Urothelial cell carcinoma of the bladder is a heterogenous group of tumors with varying malignant potential and natural history. Approximately 80% of bladder cancers are low- or intermediate-grade superficial tumors.[1] Although superficial tumors can be resected transurethrally, these tumors are characterized by a high risk of recurrence (60–85%), with maximal incidence during the first year.[2] Despite this risk, the 5-year survival rate is as high as 80–90%.[3] Therefore, the greatest concern in patients with superficial bladder cancer is not to reduce mortality but rather to lower and postpone the number of recurrences and to prevent progression to invasive disease. Consequently, long-term follow up is required.

Cystoscopy and cytology are routinely used for the diagnosis and follow up of superficial bladder tumors. Currently, cystoscopy is the most efficient method available for detecting primary or recurrent urothelial cancer (UC) of the bladder. However, cystoscopy is invasive and causes significant discomfort to the patient, and flat tumors or carcinoma *in situ* may be difficult to detect.[4] Therefore, it is important to find a test to use as a non-invasive adjunct to standard diagnostic and surveillance techniques.[5] The test must be clinically useful and easy to perform, have minimal requirements for sample preparation and handling, and be reliable[6] through high sensitivity and specificity. Urine cytology is non-invasive and has recently become the gold standard for diagnosing high-grade urothelial lesions, with sensitivity of up to 95% and specificity close to 100%. However, its sensitivity is low in low-grade tumors, which are the most common type of UC.[4,7,8]

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The limitations of cytology and cystoscopy for making the primary diagnosis and monitoring patients led to the development of new urine tests for the early detection of transitional cell carcinoma.[4] Liquid-based cytology has been developed as an alternative to conventional cytological preparations. Most comparative studies have shown that ThinPrep (TP; Cytyc, Boxborough, MA, USA) is better than conventional preparations, as it has sensitivity and specificity exceeding 90% in non-gynecological specimens.[7] MonoPrep2 (MP; MonoGen, Vernon Hills, IL, USA), a recently-developed liquid-based cytology method, uses a manual filtration system and is simple and cost effective.

This study compares MP and TP and shows that they provide smears of comparable quality and give similar diagnostic results.

Materials and methods

This study examined 284 urine samples collected consecutively between January 2003 and June 2004. Each sample was divided in half. Each half of the specimen was concentrated by centrifugation at 600 *g* for 10 min. One half was processed using the TP technique and the other by the MP technique.

The TP method uses the ThinPrep 2000 system and was designed to improve both sample collection and cytopreparation compared with conventional cytological techniques.

The MP technique involves placing a nylon mesh in front of a filter, spraying the nylon mesh and filter with Merkofix (Merck, Darmstadt, Germany), and using MonoSol C, a modification of MonoSol B. All the specimens were processed using the manufacturer's standard procedure, with the addition of the nylon mesh ($\phi = 20$ mm) placed in front of the filter to eliminate mucus. An MP filter assembly was attached to each vial. Before drawing the fluid into the plunger, the vial was agitated gently. The plunger was pulled gently to the first locked

position (10 mL of suction) and then to the second locked position (20 mL). The MP filter assembly was opened slowly, and Merkofix was sprayed on the nylon mesh and filter to prevent air-drying artifacts. The mesh was removed gently, and the upstream side of the filter was transferred to a clean, positively-charged glass slide using forceps. After 30 sec, four drops of MonoFix were placed on top of the filter and left for 30 sec for fixation. Then, a thick pad was placed over the filter and blotted using firm, even pressure over the entire filter to assure cell transfer to the slide by absorbing excess fluid. After at least 1 min, the filter was removed carefully. Then, each slide was put in 95% ethanol for a minimum of 15 min.

The TP and MP slides were stained with Papanicolaou's stain using an Autostainer (Sakura, Tokyo, Japan) and overlaid with a Coveraid (Sakura).

To reduce observer bias, the evaluator was blinded to the fact that matched slides were being read. Each cytological diagnosis was classified as 'unsatisfactory', 'benign' (negative and benign atypia), 'borderline', or 'malignant' (suspicious and transitional cell carcinoma). We also evaluated specimen quality, including the cellularity and degree of obscuring factors, such as inflammation and blood.

Subsequent biopsies were performed in 73 of the 284 patients within 2 weeks of the cytological test. For the biopsy specimen, the diagnosis was classified as benign or malignant. The diagnostic accuracy of the two cytological techniques was determined by comparison with the biopsy diagnosis.

Statistical analysis was performed with the Statistical Analysis System version 8.1 (SAS Institute, Cary, NC, USA) using the χ^2 test. A P-value of less than 0.05 was considered statistically significant.

Results

Both the MP and TP methods resulted in a uniform cellular spread in a thin layer with no cellular overlap or obscuring elements. Both preparations gave an evenly dispersed smear, with groups of crowded cells in a limited number of cases. MP and TP both produced a true monolayer, with all cells spread in the same plane of focus, requiring minimal adjustment of the fine focus during examination. Considering all the cases, both the MP and TP preparations had relatively clean backgrounds, with enhanced cellular preservation, few obscuring inflammatory cells, and little proteinaceous debris or red blood cells (RBC) casts. The cells in both the TP and MP preparations appeared rounded and showed enhanced nuclear detail. Benign urothelial cells seemed to have a greater tendency to cluster together. On cytological examination, the features of malignancy included markedly increased cellularity, cell-in-cell arrangements, and pleomorphic nuclei. Considering all the features reviewed, the overall quality of the slides produced by both techniques was similar in the majority of cases (Fig. 1).

The cytological diagnoses for the 284 cytological samples are summarized in Table 1. The rate of completely satisfactory specimens was 100% with TP and 98.6% with MP. Excluding four unsatisfactory samples, 269 of the remaining 280 samples (96.1%) showed cytological concordance that represented absolute agreement in the diagnoses based on the two techniques. The cytological diagnoses for the 44 benign and 29 malignant samples revealed on biopsy are described in Tables 2 and 3. The rate of cytological diagnosis of each condition was similar for both methods.

The overall sensitivities of MP and TP were 58.6 and 62.0%, respectively, and the specificities were 100 and 97.7%, respectively. The sensitivity and specificity did not differ significantly between the two techniques ($P > 0.05$; Table 4).

Discussion

Urine cytology comprises a large proportion of non-gynecological specimens. Although filter techniques result in better cell preservation and greater cell recovery, cytocentrifugation is used more widely in the routine cytology laboratory. However, there is concern that significant amounts of cellular material are lost during the cytocentrifugation process, thereby jeopardizing the accuracy of the diagnosis.

Accordingly, in order to compensate for this conventional cytology method, the TP processor was introduced for preparing monolayer smears for gynecological and non-gynecological cytological examinations.[9] It uses a liquid-based collection system to prepare thin-layer slides.[10] Since its introduction, TP has enjoyed favorable evaluations in a number of studies involving both gynecological and non-gynecological specimens.[11–13] In the vast majority of reports, the authors have mentioned benefits such as increased cellularity, lack of obscuring background material, improved morphology, and a decreased rate of unsatisfactory or suboptimal specimens relative to conventional cytopreparatory techniques (direct smears and cytospun specimens). Papillo and Lapen[14] compared the cell yield for a number of non-gynecological specimens using TP and the cytocentrifugation method, demonstrating increased cell recovery using the TP technique, particularly for cytological specimens with low cellularity. Beech *et al.*[15] compared 70 urine specimens prepared using the TP method and the Shadon Cyto-Spin II. In their study, the number of diagnostic cells was increased on TP slides. Non-cellular background elements were equivalent in terms of debris and casts, but erythrocytes and crystals were markedly reduced. In addition, there were fewer non-diagnostic samples with the TP method. These findings were reproduced in our study.

In recent years, liquid-based cytology has emerged as an alternative to conventional cytology. Many laboratories have successfully applied this technique to body fluids (e.g. urine, pleural effusions). Most studies report better results using the TP system compared with conventional specimens, and the residual material within the vial can be used for immunohistochemical or other analyses.[16] In Korea, TP was introduced in late 1999 because it required an expensive instrument and the specimen preparation costs were higher. This prohibits its use in routine screening for public health purposes. Therefore, there is a need for a cheaper, alternative technique that will be of practical use in screening for bladder cancer. MonoPrep2 is a recently-developed manual filtration system that is claimed to provide evenly distributed monolayers of cells, but it has not yet been evaluated by the Food and Drug Administration (FDA) for use in gynecological and non-gynecological cancer screening. Nam *et al.*[17] compared a modified MP liquid-based cytology test with a TP Papanicolaou test. They demonstrated that the modified MP method gave comparable results to those of TP for cervical cancer screening. Therefore, we reviewed the usefulness of liquid-based preparation in urine cytology.

ThinPrep and MonoPrep2 have their own cytological artifacts related to the methods of preparation. One problem with MP has been the low-volume cell yield. We have improved the yield by using Mono-Sol C in the collection vial. Another problem with MP is its inability to completely eliminate inflammatory cells. Although the inflammatory cells were not removed completely in our modified method, we reduced the number sufficiently so as not to affect the diagnostic evaluation. The TP technique is known to cause cellular shrinkage and dense staining intensity with prominent nucleoli, even in benign cells, probably as a result of the methanol-based preservative.[18] We did not see this artifact using MP in our study.

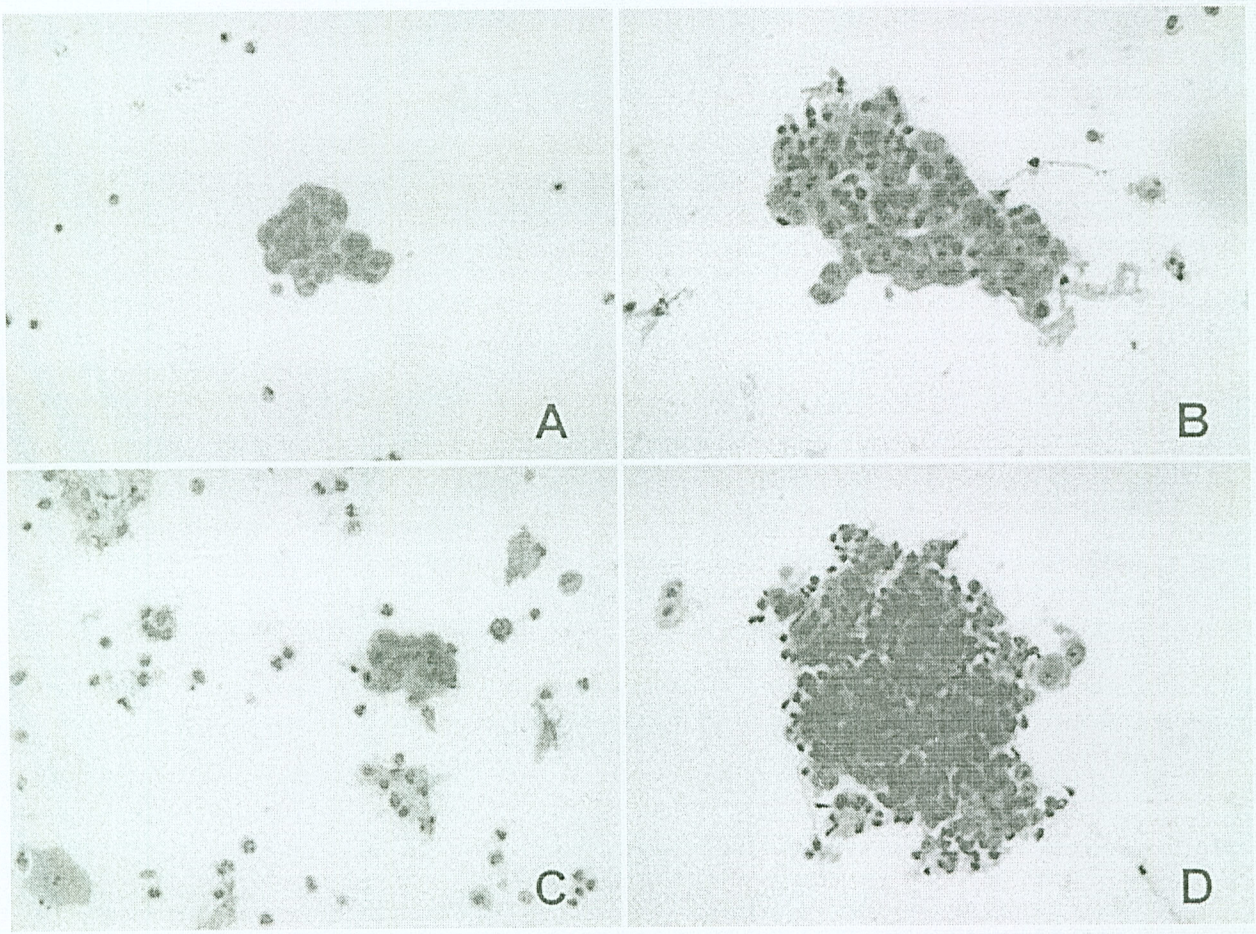

Fig. 1 Representative examples of Thin Prep (A, B) and MonoPrep (C, D). A, C: benign (×1000, Papanicolaou stain); B, D: malignant (×1000, Papanicolaou stain).

Table 1 Comparison of the cytological diagnoses between the MonoPrep2 and ThinPrep methods in 284 specimens

Diagnosis	MonoPrep2		ThinPrep	
	No. of cases	(%)	No. of cases	(%)
Unsatisfactory	4	(1.4)	0	(0)
Negative	62	(21.8)	62	(21.8)
Benign atypia	162	(57.1)	164	(57.8)
Borderline	12	(4.2)	15	(5.3)
Suspicious	27	(9.5)	27	(9.5)
TCC	17	(6.0)	16	(5.6)
Total	284	(100)	284	(100)

TCC, transitional cell carcinoma.

Table 2 Comparison of the cytological diagnoses between the MonoPrep2 and ThinPrep methods in 29 malignant specimens

Diagnosis	MonoPrep2		ThinPrep	
	No. of cases	(%)	No. of cases	(%)
Unsatisfactory	1	(3.5)	0	(0)
Negative	2	(6.9)	2	(6.9)
Benign atypia	6	(20.6)	6	(20.6)
Borderline	3	(10.4)	3	(10.4)
Suspicious	9	(31.0)	12	(41.5)
TCC	8	(27.6)	6	(20.6)
Total	29	(100)	29	(100)

In both TP and MP methods, the cell suspension is first gently dispersed, homogenizing the cell population. Therefore, the reexamination of the unfiltered cells in the vial would not be necessary to detect remnant cancer cells.[19]

In this study, the diagnostic accuracy of MP was similar to that of TP. The rates of cytological diagnoses did not differ between the MP and TP methods. In addition, both MP and TP had a low rate of unsatisfactory results.

Table 3 Comparison of the cytological diagnoses between the MonoPrep2 and ThinPrep methods in 44 benign specimens

Diagnosis	MonoPrep2		ThinPrep	
	No. of cases	(%)	No. of cases	(%)
Unsatisfactory	0	(0)	0	(0)
Negative	11	(25.0)	10	(22.7
Benign atypia	32	(72.7)	32	(72.7)
Borderline	1	(2.3)	1	(2.3)
Suspicious	0	(0)	1	(2.3)
TCC	0	(0)	0	(0)
Total	44	(100)	44	(100)

Table 4 Sensitivity and specificity of MonoPrep2 and ThinPrep for 73 patients in which a subsequent biopsy was performed

	MonoPrep2™	ThinPrep®	*P*-value*
Sensitivity	58.6%	62%	>0.05
Specificity	100%	97.7%	>0.05

*Based on χ^2 test.

Conclusions

The currently used TP methods require expensive basic equipment and disposables, resulting in increased costs, whereas MP is a simple, manual method and does not require expensive instruments for slide preparation. We found that the MP test results were comparable to those of the TP test. Therefore, we recommend MP as an alternative method for liquid-based cytology to be used for bladder cancer screening.

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