



A Comparison Between ThinPrep Monolayer and Cytospin Cytology for the Detection of Bladder Cancer

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Purpose: The sensitivity of urine cytology is higher for carcinoma in situ and poorly differentiated tumors in bladder cancer, while being fairly low for low-grade or well-differentiated tumors. Development of a sensitive diagnostic test to detect bladder carcinoma would significantly facilitate patient management and allow earlier treatment of this disease. This study compared ThinPrep urine cytology (Cytoc Co.) and conventional Cytospin urine cytology (Shandon Scientific Ltd.) in the diagnosis of bladder cancer.

Materials and Methods: From January 2002 to December 2010, ThinPrep cytology and conventional urine Cytospin cytologic examination of bladder washings were performed in 3,085 subjects suspected of having bladder cancer and in 379 patients with follow-up after transurethral resection of bladder tumor (TUR-BT). The sensitivity and specificity of the urine ThinPrep test was compared with that of conventional Cytospin cytology according to tumor number, size, pathological stage, grade, and recurrence.

Results: Of 3,085 subjects, bladder cancer was confirmed by TUR-BT in 379 subjects. The overall sensitivity of ThinPrep and Cytospin cytology was 60.9% and 59.9% in patients suspected of having bladder cancer, respectively. The overall specificity of ThinPrep and Cytospin cytology was 94.8% and 95.3% in patients suspected of having bladder cancer, respectively. The sensitivity of ThinPrep and Cytospin cytology was increased with increasing number, size, pathological stage, and grade, but there was no significant difference between the two tests.

Conclusions: These results suggest that ThinPrep cytology has no advantage in the diagnosis of bladder cancer of a low grade or low stage.

Keywords: Cytospin cytology; ThinPrep; Urinary bladder neoplasm

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INTRODUCTION

Urothelial carcinoma of the bladder is the eighth most common cancer in the United States [1]. But bladder cancer recurrence is very high, ranging from 40% to 85% according to different studies [2,3]. Because of the risk of recurrence, regular follow-up is required after treatment. Cytology is widely used to assess cancer cells in voided urine and bladder wash material. Urinary cytology is the direct microscopic investigation of shed urothelial cells. Since it was first described in 1945, it has continued to be used for the detection of neoplastic cells in the urine [4]. Cytospin and

ThinPrep cytology are both used to detect bladder tumors. The ThinPrep method uses a filtration process and thin-layer deposition of cells, which offers better cell preservation [5]. In the present study we compared bladder wash Cytospin cytology and ThinPrep cytology for detecting and predicting bladder tumors.

MATERIALS AND METHODS

Of the patients who visited the outpatient clinic of our medical institution during a period ranging from January 2002 to December 2010, a total of 3,085 presented with symp-

toms such as hematuria or bladder irritation and were clinically suspected of having bladder cancer. These 3,085 patients underwent urinary cytology using both ThinPrep and Cytospin. Of the 3,085 patients who were suspected of having bladder cancer, 379 had an established diagnosis of bladder cancer by transurethral resection of the bladder tumor. These 379 patients consisted of 314 men and 65 women, whose mean age was 65.4 years. The baseline characteristics of the patients are shown in Table 1.

The samples for urinary cytology were actively rinsed with saline by using a cystoscope. They were differentially placed in test bowls for ThinPrep or Cytospin analysis, ensuring that the volume of each sample was higher than 50 mL. Samples for conventional urinary cytology were centrifuged at 2,000 rpm for 10 minutes. Then, the precipitates were well mixed at a volume of approximately 2 to 3 mL. This was followed by precipitation by centrifugation at 1,000 rpm for 3 to 5 minutes (Cytospin 3, Shandon Scientific Ltd., Cheshire, UK). Following this, two layers of cell smear samples were prepared. The cell smear samples were fixed in 95% alcohol for 30 minutes and were then stained by using Papanicolaou dye. The ThinPrep test was performed by using an automated liquid-based monolayer cell preparation system (ThinPrep 2000 system; Cytoc Co., Boxborough, MA, USA). Briefly, for the ThinPrep test, the samples were obtained and then immersed in Cytolyt, a buffered preservative solution. Then, they were transferred to a PreservCyt bowl. The cylinder with a filtration membrane was then placed in this bowl. This was followed by rotation of the cylinder to ensure that the cells contained in the solution were homogeneously distributed. Using the negative pressure, the erythrocytes and mucus were allowed to penetrate the filtration membrane. Thus, only the cell membranes were left on the filtration membrane for the diagnostic procedure. This maneuver was repeated until an appropriate number of cells (2,000–50,000) was

collected. Thus, the urinary flow rate was adjusted. Thereafter, the cylinder was removed from the bowl. The cells left on the filtration membrane were attached to the slide and then fixed, for which 95% alcohol was used a fixative as done for Cytospin urinary cytology [6]. Based on the test results, both regimens adopted criteria for positive results such as positive for malignancy, suggestive of malignancy, and atypical cell presentation. In addition, the clinical characteristics included the degree of histological differentiation, T-stage (Ta-1 or > T2), size (< 1, 2–4, and > 4 cm), number (1, 2–3, and > 4), and recurrence, based on which we compared the sensitivity and specificity of the two methods. The staging was done on the basis of the TNM classification system proposed by the American Joint Committee on Cancer. In addition, the degree of differentiation was based on the International Society of Urologic Pathologists consensus classification system. Statistical analysis was done by using the chi-square test. A p-value of < 0.05 was considered statistically significant.

RESULTS

In the 3,085 patients who were suspected of having bladder cancer, the sensitivity of urinary cytology based on the ThinPrep and Cytospin tests was 60.9% and 59.9%, respectively. In addition, the specificity was 94.7% and 95.2%, respectively. These results indicate that there were no significant differences between the two tests (Table 2). Of the 379 patients who were followed up after transurethral resection of the bladder cancer, 314 were monitored for their clinical course. In these patients, the sensitivity and specificity of both regimens were 26.2% and 92.3%, respectively (Table 3). The sensitivity of ThinPrep and Cytospin urinary cytology depending on the number of tumors was 48.0% and 49.0% for one tumor, 66.2% and 63.1% for 2 to 3 tumors, and 82.9% and 85.4% for more than four tumors, respectively. These results indicate that there were no significant differences between the two tests (Table 4).

TABLE 1. Baseline characteristics of the 379 patients with an established diagnosis of bladder cancer by transurethral resection of the bladder tumor

Parameter	Value
Sex	
Men	314
Women	65
Age (y), mean (range)	65.4 (43–78)
Cancer	
Size (cm)	
< 2	182
2–4	97
> 4	66
Grade	
Low	241
High	104
Stage	
Ta–T1	278
≥ T2	67

TABLE 2. Sensitivity and specificity of ThinPrep and Cytospin urinary cytology in patients suspected of having bladder cancer

Parameter	ThinPrep	Cytospin	p-value
Sensitivity	231/379 (60.9)	227/379 (59.9)	0.320
Specificity	2,563/2,706 (94.7)	2,575/2,706 (95.2)	0.318

Values are presented as number (%).

TABLE 3. Sensitivity and specificity of ThinPrep and Cytospin urinary cytology in patients followed up

Parameter	ThinPrep	Cytospin
Sensitivity	11/42 (26.2)	11/42 (26.2)
Specificity	311/337 (92.3)	311/337 (92.3)

Values are presented as number (%).

TABLE 4. Sensitivity of ThinPrep and Cytospin urinary cytology according to the number of bladder tumors

No. of bladder tumors	Sensitivity, n (%)		p-value
	ThinPrep	Cytospin	
1 (n=198)	95 (48.0)	97 (49.0)	0.321
2-3 (n=65)	43 (66.2)	41 (63.1)	0.326
≥ 4 (n=82)	68 (82.9)	70 (85.4)	0.324

TABLE 5. Sensitivity of ThinPrep and Cytospin urinary cytology according to the size of the bladder tumor

Size (cm)	Sensitivity, n (%)		p-value
	ThinPrep	Cytospin	
< 2 (n=182)	81 (44.5)	77 (42.3)	0.321
2-4 (n=97)	68 (70.1)	68 (70.1)	0.67
> 4 (n=66)	62 (93.9)	62 (93.9)	0.78

We also compared the sensitivity depending on the size of the tumor. The sensitivity of ThinPrep and Cytospin urinary cytology was 44.5% and 42.3% in cases with a tumor size < 2 cm, 70.1% in cases with tumor size of 2-4 cm, and 93.9% in cases with tumor size > 4 cm. This showed no significant difference (Table 5). In addition, we compared the sensitivity depending on the TNM stage between ThinPrep and Cytospin urinary cytology. This also showed no significant differences (Tables 6 and 7).

DISCUSSION

Bladder cancer is one of the most common urological cancers in Korea. Most cases of bladder cancer are transitional epithelial cancer. In addition, superficial bladder cancer accounts for approximately 70% of the total cases [7].

Bladder cancer is characterized by gross hematuria. An early diagnosis of bladder cancer can be made by using cystoscopy and urinary cytology. Approximately 80% of total cases show recurrence after treatment. In approximately 10% to 25% of the cases of recurred cancer, the degree of differentiation and the TNM stage get worse. After treatment, follow-up examination should be performed by using urinary cytology and cystoscopy [8].

Urinary cytology was first attempted to make a diagnosis of bladder cancer by using microscopic examination of the urine deposits in 1892. Thereafter, in 1945, Papanicolaou and Marshall [4] introduced a diagnostic procedure. Since then, several authors have actively examined the method. Up to the present, urinary cytology has been used as a key modality for the diagnosis and follow-up examination of bladder cancer [9-11]. In particular, urinary cytology is useful for performing a mass screening of a high-risk group of patients, allowing the diagnosis of intraepithelial cancer

TABLE 6. Sensitivity of ThinPrep and Cytospin cytology according to the stage of the bladder tumor

Stage of the bladder tumor	Sensitivity, n (%)		p-value
	ThinPrep	Cytospin	
Ta-T1 (n=278)	146 (52.5)	144 (51.8)	0.320
≥ T2 (n=67)	64 (95.5)	64 (95.5)	0.740

TABLE 7. Sensitivity of ThinPrep and Cytospin cytology according to the grade of the bladder tumor

Grade of the bladder tumor	Sensitivity, n (%)		p-value
	ThinPrep	Cytospin	
Low (n=241)	129 (53.5)	127 (52.7)	0.318
High (n=104)	93 (89.4)	93 (89.4)	0.670

and detecting cancer in the bladder diverticulum.

Foliated bladder cancer cells are characterized by hyperchromatic, irregularly shaped nuclei and an increased nucleus-to-cytoplasm ratio. In cases of superficial bladder cancer with a high degree of differentiation, however, it is difficult to detect the abnormal cytology from the bladder irrigation. The detection of abnormal cytology is subject to technical expertise, the frequency of sample collection, and the criteria used to interpret the results [12-14]. Factors that may affect the results of urinary cytology include the delayed transfer of samples, the presence of urinary tract infection, radiotherapy, intravesical drug therapy, and anticancer chemotherapy. To enhance the accuracy of urinary cytology, the samples should be fixed and then transferred to a laboratory immediately after they are collected. In addition, the cytopathologists should be given all the clinical data of the corresponding patients. There is variability in the sensitivity of urinary cytology depending on the observer in patients with bladder cancer; the sensitivity has been reported to be 40% to 70% in voided urine and somewhat higher in the bladder irrigation. Thus, it has been reported that the sensitivity is relatively higher in cancer with a greater number, a larger size, a higher rate of recurrence, an advanced stage, and a lower degree of differentiation [15-17]. Our results also showed that the sensitivity was increased to 49% in cases of one cancer, to 63.1% in cases of two cancers, and to 85.4% in cases of more than four cancers. In addition, the sensitivity was increased from 42.3% in cases of tumor size < 2 cm to 70.1% in cases of tumor size of 2 to 4 cm and 93.9% in cases of tumor size > 4 cm. Moreover, it was 51.8% in low-grade cancer but 95.5% in high-grade cancer. Furthermore, it was 52.6% in well-differentiated cancer and 89.5% in poorly differentiated cancer. These findings are consistent with previous reports.

In the urine ThinPrep test based on the liquid-based

monolayer cell preparation system, the collection instruments are washed in a small bowl containing Cytolyt, a buffered preservative solution. This is followed by processing with the ThinPrep Processor. Thus, clustered cells are dispersed. Any constituents that may interfere with the diagnostic procedure, such as blood, mucus, and inflammatory cells, are removed. Only the diagnostic cells can be selectively harvested by using a specialized filter. This leads to the formation of a homogeneous group of a single layer of cells with a thickness of 2 cm that are smeared on the glass slide. The urine ThinPrep test is advantageous because the samples are collected by rinsing them in a preservative without directly smearing them on the glass side. Thus, all the diagnostic cells can be collected, although 80% of them have been formerly discarded in a collection instrument. In addition, it is also advantageous for preventing the deformity of cells due to drying immediately after they are collected. In addition, it is advantageous in that a single layer of cells at a thickness of 2 cm is consistently smeared on the glass slide. This makes it easier to interpret the results and thereby reduces the difficulty in interpreting the results because of clustering, overlapping, and deformation of the cells, blood, and infections [18]. Owing to these advantages, the ThinPrep test is widely used to diagnose cancer in other specialty areas. Cheung et al. [19] performed a mass screening with the use of the ThinPrep test and a conventional method in a total of 191,581 patients and reported that the rate of detection of squamous cell carcinoma was 0.005% and 0.001% with the two methods, respectively. This indicates that the diagnostic value of the ThinPrep test was significantly higher. Negri et al. [20] compared the results of the ThinPrep test, conventional cytology, and histopathologic examination in 214 patients with an established diagnosis of uterine cervix cancer and reported that the sensitivity was 50.9% and 43.9% in the corresponding order. This indicates that the ThinPrep test showed excellent results. In the current study, however, the sensitivity of ThinPrep urinary cytology and conventional urinary cytology was 60.9% and 59.9%, respectively, in 379 patients with an established diagnosis of bladder cancer. This indicates that there was no significant difference in sensitivity between the two tests. In the 42 patients with recurrence, the sensitivity was also 26.2%. That is, there was also no significant difference in the sensitivity. Moreover, compared with the conventional methods based on Cytospin, the ThinPrep test showed no significant difference in the sensitivity depending on the number, size, TNM stage, or degree of differentiation of the tumor. Another study [21] compared the results between the ThinPrep test and conventional cytology in 184 samples with an established diagnosis of bladder cancer or benignity and reported that the results of the two tests were very similar. No significant differences in the cytomorphometric parameters were measured by digital image analysis.

CONCLUSIONS

In the current study, we compared the number, size, TNM stage, the degree of differentiation, and the recurrence of bladder cancer between ThinPrep urinary cytology and conventional Cytospin urinary cytology for diagnosing bladder cancer. Our results showed that there were no significant differences in sensitivity or specificity between the two methods. Our results also showed, however, that the accuracy of urinary cytology based on ThinPrep or Cytospin cytology was relatively lower in patients with a low-grade tumor with a high degree of differentiation and in those who were followed up after transurethral resection of the bladder cancer. Therefore, cystoscopy should be used concomitantly in these patients.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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