The role of collecting bladder wash fluid before biopsy procedure to help the cytological diagnosis of residual tumor

**ABSTRACT**

**Background:** Urinary cytology has low sensitivity and specificity in urinary neoplasm.

**Aim:** We planned to assess whether the examination of bladder washing before biopsy (WBB) plays a role in better cytologic diagnosis of bladder wash fluid collected after biopsy procedure (WAB) in papillary urothelial neoplasms.

**Materials and Methods:** We included 36 patients with papillary lesion of bladder. Prior to the biopsy, the bladder is washed and fluid is collected for cytology; later transurethral resection (TUR) is performed, then bladders are washed again and the fluid is separately collected for cytology. Both fluids were centrifuged and stained with May-Grünwald Giemsa (MGG). First the WAB slides were evaluated and diagnosed. After evaluation of the WBB slides, the WAB slides were rediagnosed. Presence of cellularity, papillary structure, fusiform cells, background bleeding, and cytolysis in WBB and WAB were evaluated separately.

**Results:** We determined that 31 WBB samples were hypercellular, and 12 of them remained as hypercellular in WAB. Papillary structures were observed in 20 WBB samples; and in one WAB cytology. In 29 cases where no fusiform cells are identified in WBB, 22 showed fusiform cells in WAB. Cytolysis in WABs was noted in 15 cases whose WBBs did not show cytolysis. The decrease in cellularity, papillary structure ($P < 0.001$, both), cytolysis ($P = 0.008$), and fusiform cells ($P < 0.001$) were statistically significant. After seeing the WBB slides, we reevaluated the WAB slides. Out of the eight out of 36 (22.2%) samples diagnosed with degeneration previously, five (62.5%) samples were rediagnosed as benign, two (25%) as cytologic atypia which favor reactive, and one (12.5%) as malignant.

**Conclusion:** Due to the better quality, initial evaluation of WBB may help more effective diagnoses of WAB slides.

**Key words:** Bladder; urine cytology; washing

**Introduction**

Urothelial carcinoma (UC) of the bladder is the 7th most common cancer.\[1\] The combination of urinary cytology and cystoscopy are currently accepted procedures for the diagnosis and follow up of bladder tumors and urinary tract malignancies.\[2-5\] The main goal of cytological examination during initial diagnosis and in follow-ups of bladder cancer is to minimize mortality and treatment costs.

With the recent improvements in flexible endoscopy, bladder cells are managed better and the number of diagnostic cellular elements are higher in bladder wash fluid (BW) than voided urine samples; therefore the quality of cytology obtained using BW is better than voided urine samples.\[6\]

Although the sensitivities of urinary-based markers and techniques [BTA, nuclear matrix protein, (NMP)-22, Immuno Cyt, Fluorescent In Situ Hybridization (FISH), telomerase, microsatellite analysis, BLCA-4, fibrin, fibrinogen degradation, and hyaluronic acid products] are high, they still don’t have enough specificity to replace routine urinary cytology for the diagnosis and follow-up of urothelial neoplasms.\[7,8\] One review of 36 studies containing 14,260 patients reported the sensitivity and specificity of urinary cytology to be 44% and 96%, respectively.\[9\]
Urinary cytology is not very effective in detecting low-grade lesions while it is more suitable for detecting carcinoma in situ. The main disadvantage of low-grade lesions is its low sensitivity in urinary cytology. The experience of the pathologist is also important. Studies show that the sensitivity of urinary cytology is higher in larger centers with more experienced pathologists than in the non-academic small hospitals with less experienced ones. These studies indicate that the differences among observers are significant and training of pathologist remarkably increases their ability for more accurate diagnosis. In addition, the formation of reactive cytological changes due to urinary stones, infections and previous treatments can be the source of misdiagnosis.

Shin et al. showed that the stage, grade, size, multifocality, and recurrence of bladder tumors were statistically higher in the patients with positive urinary cytology performed after transurethral resection of non–muscle-invasive bladder cancer. The same study also indicated that bladder cytology is an independent prognostic factor to predict cancer recurrence. Every factor, which may help in better evaluation of bladder cytology, is vitally important for patients.

Bladder washing after transurethral biopsy is performed to rule out residual tumors or carcinoma in situ after excision of a papillary lesion. Bladder wash fluid collected before biopsy (WBB) is done to visualize the bladder. Our aim is to compare the cytology of WBB and bladder wash fluid collected after biopsy (WAB) to find the possible role of this comparison in determining the residual tumor in WAB cytology.

**Materials and Methods**

Between April 2011 and February 2012, 36 patients with papillary lesions of bladder were included in the study. Fifteen (41.6%) patients were diagnosed with a new papillary lesion and 21 (58.3%) patients were already being followed up for papillary urothelial carcinoma (PUC). Cystoscopy of the patients was performed using a 21F rigid cystoscope placed transurethrally, under general anesthesia, and the bladder was inspected systematically. The site, size, appearance, and number of tumors were determined during the cystoscopy. In addition, bladder was washed with pressured 0.9% saline using a 50 mL syringe attached to the cystoscope and samples were collected (designated as WBB) for urinary cytology. Subsequently, the cystoscopy was removed and a 26F resectoscope was transurethrally inserted into the bladder and all tumor tissues were removed via transurethral resection (TUR) approach. After taking out the tumor tissues, additional TUR was applied to the floors of the resected tumors, and then deep muscle tissue samples were picked up before cauterizing tumor resection sites. After ensuring that no tumor tissue remained, the bladder was washed with pressured 0.9% saline using a 50 mL syringe attached to the cystoscope and samples were collected from bladder wash fluid (designated as bladder wash fluid collected after biopsy (WAB) for urinary cytology. Bladder wash fluids (20-50 mL) were centrifuged at 1800 rpm for 6 min and the supernatants residing at the bottom-third of the tubes were collected for preparing cytological slides. The slides were stained with May-Grünwald Giemsa (MGG). Initially, two pathologists (AK: Aydan Kılıçarslan, NS: Nuran Süngü) were asked to make their diagnosis for the slides prepared from WAB. The degree of cellularity (hypocellularity was number of clusters or groups less than five, hypercellularity was more than five), presence of papillary structure, fusiform cells owing to cauterization artifact, background bleeding, and cytolysis were noted. The McNemar test was used to compare the changes in the proportions of characteristics. Afterwards, the pathologists reassessed their diagnoses for WAB slides after evaluating WBB slides. The cases with changed diagnoses are further evaluated.

**Results**

Bladder tissue biopsies of the patients were classified according to the 2004 WHO/ISUP classification. Among the 36 patients, 9 (25%) were identified as benign, 2 (5.5%) were PUNLM (papillary urothelial neoplasm of low malignant potential), 12 (33.3%) were non-invasive low grade urothelial carcinoma, 3 (8.3%) were non-invasive high grade urothelial carcinoma, and 10 (27.7%) were invasive urothelial carcinoma.

The presence of cellularity, papillary structure, fusiform, background bleeding, and cytolysis were compared in the WBB and WAB of all the cases [Figure 1].

We observed that 31 out 36 (86.1%) cases were hypercellular in WBBs. Twelve of 31 (38.7%) retained their hypercellularity in WAB, while 19 (61.3%) turned out to be hypocellular. Among the 5 of those that were hypocellular in WBB, 4 remained hypocellular and 1 became hypercellular. Overall, the cellularity was decreasing in WAB compared to WBB slides (McNemar test; $P < 0.001$).

WBBs of 20 out of 36 (55.6%) cases showed papillary structures and no papillary structure was noted in 19 (52.8%) of the corresponding WABs; one case still had the papillary structure in WAB. Sixteen (44.4%) patients presenting no papillary structures in the previous WBB, did not show papillary structure in WAB as well. The presence of papillary structures in WABs of these patients was lower than those
of their WBBs (McNemar test; \( P < 0.001 \)).

Furthermore, while no widespread cytolysis was observed in WBBs, prominent cytolysis was present in urothelial cells of WABs [Figures 2 and 3]. Twenty-nine (80.6%) cases did not have cytolysis in WBB, and a corresponding fifteen (51.7%) had cytolysis in WAB. Seven (19.4%) cases initially had cytolysis in WBB; and four (57.1%) of the corresponding still had cytolysis in WAB. The presence of cytolysis in WABs was higher than that of WBBs (McNemar test; \( P = 0.008 \)).

Numerous fusiform cells mostly owing to cauterization artifact were present at the background of WAB [Figure 4]. In fact, while no fusiform cells were present in WBBs of 29 (80.5%) patients, fusiform cells were detected in 22/29 of them. Besides, fusiform cells in WABs were still observed in 4 of 7 patients whose WBBs contained fusiform cells. The presence of fusiform cells in WABs were higher than that of WBBs (McNemar test; \( P < 0.001 \)).

There was no difference between WBBs and WABs in terms of erythrocytes in the background (McNemar test; \( P = 0.500 \)).

Cytology slides from WABs were evaluated simultaneously by two pathologists (AK, NS). According to the assessment of the slides, 8 (22.2%) patients were diagnosed with degeneration, 21 (58.3%) patients with benign cytology, 2 (5.5%) cases with cytologic atypia that favor reactive changes (CAWFR), 2 (5.5%) patients with cytologic atypia which favor neoplasia (CAWFN), and 2 (5.5%) patients with malignant cytology [Table 1]. Later, WAB cytology slides were reviewed and compared with WBB slides. Twenty-five (69.4%) patients were diagnosed with benign cytology, 6 (16.6%) cases showed CAWFR, 2 (5.5%) patients were diagnosed with CAWFN, and 3 (8.3%) patients were interpreted to have malignant cytology.

There was conflict in 8 (22.2%) cases. Eight of the prior diagnoses of degeneration were changed into benign in 5 (62.5%) cases, cytologic atypia favor reactive in 2 (25%) cases, and malignant in 1 (12.5%) case. Two benign cases were rediagnosed as reactive atypia [Figure 5]. Other diagnoses remained the same.

Discussion

Urinary cytology is one of the most important methods used in the urothelial neoplasm management. Urethelial carcinoma (UC) follow-up protocols suggest cytologic examinations every three months for 1-3 years; later every six months or yearly.\(^{[14]}\) While sensitivity and specificity of urinary cytology are higher in high-grade UC, they are lower in low-grade UC.\(^{[15]}\) Low interobserver and intraobserver reliability rate limits the broad use of urinary cytology.\(^{[16,17]}\) Therefore, this setback of urinary cytology has triggered the development of urinary-based tumor markers. While these tumor markers possess higher sensitivities in the diagnoses of UC, their specificities are lower with respect to the urinary cytology.\(^{[9,18]}\) Besides, false-positive rates for these tumor markers are between 15-20%.\(^{[4,8,9]}\) False-positive rate for urinary cytology...
To determine if this baseline picture would help the cytologic diagnosis, a reevaluation of WAB samples was made after examining WBB samples. We found that the diagnosis was changed in 8 cases which were diagnosed as degeneration when only WAB slides are assessed. When these cytologies were reevaluated using WBB and WAB slides together, 4 of them were determined to have benign cytology, 2 had cytologic atypia which favor reactive, and 1 was diagnosed with malignant cytology. These observations indicate that evaluation of WAB slides together with WBB slides allowed us to interpret cytological findings in WAB slides better. In addition, the interpretation of one patient with malignancy and two patients with cytologic atypia which favor reactive indicated that this approach facilitated the diagnoses of cytologic atypia. Although the number of the patients included in the present study is low, the accurate diagnosis of malignant cytology for one more patient is still valuable, and this approach indicated that the evaluation of WBB and WAB slides together has the potential to increase the sensitivity of urinary cytology.

Moreover, when 21 cases diagnosed with benign cytology during the assessment of WAB slides were reevaluated with WBB sides, 2 cases were determined to have cytologic atypia which favor reactive, indicating that incorporation of WBB slides to examination of WAB slides facilitated the distinction of reactive cytologic atypia from benign cytology. However, this approach did not notably affect the accuracy of the diagnoses made for the cases with malignant cytology, cytologic atypia which favor reactive and neoplasia.

In the literature, we encountered no studies examining the contribution of cytology slides prepared from the bladder wash fluid before biopsy.
fluids before excision of papillary lesions to the accuracy of final cytological diagnosis. The tumor markers that have been reported in the literature in recent years are relatively expensive and not widely used in the routine practice. On the other hand, preparation of cytology slides is easy and cheap. We suppose that the integration of cytology slides prepared from WBB to routine assessments of WAB slides would increase the accuracy of bladder wash fluid cytology diagnoses. Consequently, this simple approach can increase specificities and sensitivities of cytology diagnoses and in addition can contribute to the training of cytopathologists. Since the present study is the first study for the current approach, further studies are needed to clarify the effectiveness of present approach on the enhancement of specificity rates for urinary cytology.

**Conclusion**

In conclusion, WBB reveals increased quality of samples. There is less cytolysis and fewer artifacts of cauterization. WBB helps the diagnosis and even leads to changes in the diagnosis in some situations. The results of the present study suggest that integration of this simple and cheap step to routine practice can increase accuracy of cytologic diagnoses for bladder wash samples.

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