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ORIGINAL ARTICLE

A Novel Technique to Improve the Processing of Minute Ureteroscopic Biopsies

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Abstract To examine the ability of a new specimen handling technique to improve histopathological yield of ureteroscopic biopsies, performed in patients with suspected upper tract urothelial carcinoma (UTUC). In a bi-center retrospective study we compared the results of the new tissue handling technique (group 1) with the standard technique (group 2). In the new technique, to achieve maximal tissue preservation, the specimen is mounted on filter paper prior to embedding in paraffin. Multivariate analysis was performed to determine which factors are associated with optimal histological results. We further compared the biopsies with the final specimen in a subgroup of patients who underwent nephroureterectomy (NU). Of 55 ureteroscopic biopsies, 1 biopsy from group 1 (new technique) and 3 biopsies from group 2 (standard technique) were inadequate for pathological examination. 51 UTUC specimens were analyzed. Tumor grade and stage were determined in 85% and 63% of the patients in group 1 and in 83% and 25% of group 2 (p=0.85 and p=0.007). Orientation was preserved in 82% of group 1 and 42% of group 2 (p=0.003). On multivariate analysis biopsy technique and biopsy diameter were found to predict stage determination (p=0.01 and p=0.007) and tissue orientation (p=0.005 and p=0.04). Among patients who underwent NU, stage concordance between the biopsy and final pathology was observed in 56% and 27% of the patients in group 1 and 2, respectively. The new processing technique for small UTUC forceps biopsies decreases the rate of biopsies with insufficient material and improves biopsy interpretation.

Keywords Upper urinary tract urothelial carcinoma · Ureteroscopy · Biopsy · Pathology

Introduction

Urothelial carcinoma is the most common malignancy of the urinary tract. UTUC arises from the pelvicalceal system or the ureter and account for ~5% of urothelial tumors [1]. The diagnosis of UTUC is based on a combination of radiographic, cytological and endoscopic findings. Diagnostic ureteroscopy is recommended as part of the standard evaluation of suspected UTUC [2, 3]. It allows visual inspection of the upper urinary tract and enables tissue diagnosis by biopsy. The results of ureteroscopic biopsies play an important role in treatment decision-making. Although NU is considered the gold standard treatment of UTUC, endoscopic tumor resection can now be successfully implemented in patients with small, low grade, low stage tumors. In well-selected patients, the oncological outcomes of endoscopic management are similar to those of NU with 5 years cancer specific survival of more than 90% [4–6]. Despite advances in endoscopic techniques, obtaining an adequate tissue sample for accurate histopathological diagnosis remains challenging. In previous reports, the rate of non-diagnostic ureteroscopic biopsies was more than 10% [7, 8] and the inability to identify lamina propria for UTUC staging was up to ~80% [9]. Biopsy interpretation is particularly difficult when only scant amount of tissue is

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available for pathological analysis. This is often the situation when the only way to obtain the biopsy is by using biopsy forceps. In such cases, tissue orientation is often lost and differentiating benign from malignant may not be possible. Kleinmann et al. demonstrated that using the basket technique to obtain higher biopsy volume improves the diagnostic accuracy of upper tract biopsies significantly [10]. However, not all renal lesions are amenable to a basket biopsy. Flat lesions as well as solid or small papillary lesion often do not allow trapping of the tumor between the basket wires. In such cases, the biopsy is retrieved with biopsy forceps.

To improve the pathological results of minute ureteroscopic forceps biopsies and reduce the loss of tissue samples during processing, we propose the application of a tissue handling technique, used for minute conjunctival tissue [11].

Fig. 1 The proposed technique for handling ureteroscopic biopsy. The tissue is placed on a small piece of filter paper. The biopsy is flattened on the paper, allowing the specimen to adhere to the surface

Methods

In a bi-center retrospective study, we compared the histological results of the new tissue handling technique with the standard technique in patients with renal or ureteral lesions that were not amenable to basket biopsy. The study cohort included consecutive patients who underwent diagnostic ureteroscopy and forceps biopsy of suspected UTUC at the department of urology, Rabin Medical Center (RMC), Israel and the section of urology, University of Chicago Medical Center (UCMC), USA. The institutional review board of both centers approved this study.

Biopsy Techniques

Diagnostic ureteroscopy was performed as previously described [2]. A 1.6 mm standard cup biopsy forceps (Richard Wolf) was utilized. Typically, multiple biopsies were taken from the same lesion until the surgeon estimated that the amount of grossly visible tumor is sufficient for processing. In the standard technique the specimen is placed in the sample bottle with 4% buffered formalin and sent to the pathology laboratory for routine processing.

In the new technique, ureteroscopic biopsy tissue is taken using the biopsy forceps. The tissue is removed from the forceps jaws preferentially by immersing the open forceps in a small plastic container filled with 0.9% saline solution. If the tissue is not seen clearly floating in the fluid a 21G needle is used to remove the biopsy from the forceps jaws. Finally, the biopsy is removed from the saline solution utilizing a needle and placed on a small piece of filter paper. Gently, the biopsy is flattened on the paper, allowing the specimen to adhere to the surface (Fig. 1). Following one minute, the mounted biopsy is placed in 4% buffered formalin bottle. Next, the mounted biopsy is transferred to a cassette, passed through the embedding center and embedded in paraffin. Finally, the filter paper is removed and the sample is further processed using routine histopathological techniques.

Data Analysis

All the pathology slides, included in the study, were reviewed by a single pathologist (R.K) who was blinded to the processing technique and received the final slides. The endpoints for biopsy revision were the ability to maintain the urothelium orientation and to assign tumor grade and stage. Stage was assigned, according to the AJCC TNM staging system [12], whenever lamina propria was identified in the specimen. The medical chart of each patient was reviewed to obtain demographics and tumor characteristics. Basic descriptive statistics for categorical and continuous variables are provided. Chi-square test was applied to compare the histological results with $p < 0.05$ considered significant. Multivariate logistic regression analyses were used to examine which factors are associated with the ability to determine tumor grade, stage and tissue orientation. Next, we analyzed a subgroup of patients who underwent NU and compared the results of ureteroscopic biopsy with the histology of the final surgical specimen. Analyses were performed using Stata 13 (Statacorp. College Station, TX, USA).

Results

A total of 55 patients in the two centers underwent diagnostic ureteroscopy with forceps biopsy for suspected UTUC. The filter paper technique was utilized in 28 patients (group 1) while the standard technique was used in 27 (group 2). 4 biopsies were inadequate for pathological examination, 1(3%) was from group 1 and 3 (11%) from group 2. Therefore, the final analysis included 51 patients (27 patients in group 1 and 24 in group 2).

Table 1 Clinical characteristics according to biopsy technique

Characteristic	Group 1 Filter paper technique *n* = 27	Group 2 Standard technique *n* = 24	*P-value*
Age, mean (SD)	68 (12)	69 (12)	0.9
Gender, *n* (%)			0.1
Males	24 (89)	17 (70)	
Female	13 (11)	7 (30)	
Tumor side, *n* (%)			0.58
Left	18 (67)	13 (54)	
Right	11 (33)	11 (46)	
Tumor location, *n* (%)			0.7
Kidney	12 (45)	12 (50)	
Ureter, proximal	4 (15)	7 (30)	
Ureter, distal	11 (40)	5 (20)	
Tumor diameter, mm (SD)	19 (9)	15 (7)	0.06
Final treatment approach, *n* (%)			0.4
Endoscopic	11 (41)	13 (54)	
Nephroureterectomy	16 (59)	11 (46)	

Patients and tumors characteristics according to the study groups are summarized in Table 1. No statistically significant differences in baseline characteristics were found between the two groups. A comparison between the histological results of the study groups is presented in Table 2. Tumor grade and stage were determined in 85% and 63% of the patients in group 1 and in 83% and 25% of group 2 (p = 0.85 and p = 0.007). Tissue orientation was preserved in 82% of group 1 and 42% of group 2 (p = 0.003) (Fig. 2). The average biopsy size was 3.8 mm and 4 mm in group 1 and 2, respectively (p = 0.9). On multivariate analysis biopsy technique and maximal biopsy diameter were found to predict stage determination (p = 0.01 and p = 0.007) and tissue orientation (p = 0.005 and p = 0.04). Biopsy size predicted grade determination as well (p = 0.01) (Table 3).

NU was performed in 27 patients, with an average duration of 41(±25) days between the biopsy and surgery. In 16/27 patients, ureteroscopic biopsy was obtained using the filter paper technique and in 11/27 the standard technique was utilized. The histological results of NU, according to biopsy technique, are shown in Table 4. Grade concordance between biopsy and surgical specimen was found in 69% and 64% in the filter paper group and in the standard biopsy processing group, respectively. Upgrading was observed in two patients

Table 2 Histological results of ureteroscopy according to biopsy technique

	Group 1 Filter paper technique *n* = 27	Group 2 Standard technique *n* = 24	*P-value*
Biopsy diameter, mm, mean (SD)	3.8 (1.8)	4 (1.8)	0.9
Grade determined, *n* (%)	23 (85)	20 (83)	0.85
Low grade	18	14	
High grade	5	6	
Grade undetermined, *n* (%)	4 (15)	4 (17)	
Stage determined, *n* (%)	17 (63)	6 (25)	0.007
Ta	12	5	
T1	5	0	
T2	0	1	
Stage undetermined, *n* (%)	10 (37)	18 (75)	
Orientation preserved, *n* (%)	22 (82)	10 (42)	0.003

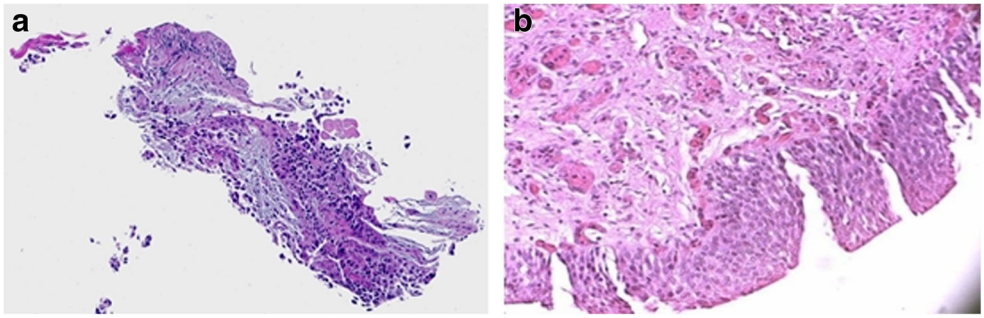

Fig. 2 Histopathology specimens obtained using the standard technique **a*** and the filter paper technique **b****; Notice tissue orientation is better preserved with the new technique **b**. *H&E X40. **H&E X100

in the filter paper group and one patient in the standard biopsy group. Stage concordance was found in 56% (filter paper) and 27% (standard) of the patients in each group. Upstaging was recorded in 4 patients from the filter paper group (Table 5).

Discussion

Although UTUC is relatively rare, its incidence in western countries has slowly risen in the last decades [13–15]. In parallel to this trend, the endoscopic approach for the treatment of UTUC has been extended from patients with imperative indications (e.g. chronic kidney disease, bilateral disease or solitary kidney) to selected patients with elective indications. Long term retrospective studies show that patients with low grade UTUC, treated endoscopically, had renal preservation rate of ~80% [16] with cancer specific and overall survival equivalent to that of NU [17–19]. However, in patients with high grade/stage UTUC, treated endoscopically, survival may be reduced. Therefore, complete and accurate pathological confirmation is of paramount importance for treatment decision making. Obtaining a good quality biopsy specimen from the upper urinary tract is challenging. Tissue samples obtained endoscopically, particularly with biopsy forceps, are usually small and fragile. Some of the minute tissues are damaged or even lost during routine histopathological processing. Furthermore, small biopsy specimens tend to form a spherical shape when placed in the specimen bottle thus changing the original structural orientation. Lack of proper tissue orientation does not allow stage determination and is commonly encountered when forceps biopsy specimens are processed [20, 21].

We present a simple and reproducible technique, which has not been used in the field of urology, for handling and processing ureteroscopic biopsies. Using this technique, we observed lower rate of specimens that were insufficient for analysis or lost during processing. Furthermore, we noticed improved preservation of tissue orientation and increased number of biopsies with stage determination. With the new technique stage could be determined in 63% of the patients in comparison to only 25% with the standard technique (p = 0.007). The concordance of the biopsy stage with the final surgical pathology was 56% which is higher than previously reported by Rojas et al. who reported 43% [22]. As expected, grade determination which is based on the cellular structure and not dependent on tissue orientation was similar between the new and standard technique.

Different techniques have been applied to improve the quality of ureteroscopic biopsies. In the multi-biopsy ureteroscopic approach, described by Guarnizo et al. at least 6 biopsies were obtained during ureteroscopy [7]. UTUC was confirmed histologically in 40/45 patients with the disease (~90%) and tumor grade was accurately determined in 78% of cases. Stage, however, was assigned in only two-thirds of biopsies with significant upstaging (37%). Although, there is no doubt that with higher number of biopsies the accuracy of the results may improve, repeated biopsies, in our experience, are often associated with more bleeding that obscures the visibility.

Table 3 Multivariate analysis of factors associated with determination of tumor grade, stage and orientation on ureteroscopic biopsy

	Grade		Stage		Orientation	
Factor	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
Age	−0.2 (−0.37, 0.02)	0.08	0.02 (−0.04, 0.08)	0.5	−0.007 (−0.06, 0.05)	0.8
Maximal tumor diameter, mm	0.08 (−0.07, 0.23)	0.3	−0.02 (−0.13, 0.09)	0.7	−0.03 (−0.1, 0.06)	0.5
Tumor location	0.1 (−1.5, 1.75)	0.9	0.45 (−0.5, 1.45)	0.3	−0.1 (−1, 0.8)	0.8
Maximal Biopsy diameter, mm	1.4 (0.28, 2.5)	0.01	0.7 (0.2, 1.2)	0.007	0.4 (0.001, 0.85)	0.04
Biopsy technique	0.6 (−1.7, 2.9)	0.6	2.4 (0.58, 4.2)	0.01	2.3 (0.7, 3.8)	0.005

Table 4 Histological results of nephroureterectomy according to biopsy technique

	Filter paper technique *n = 16*	Standard technique *n = 11*
Surgical grade, *n* (%)		
Low grade	10 (37)	4 (36)
High grade	6 (63)	7 (64)
Surgical stage, *n* (%)		
Ta	5 (31)	5 (45)
T1	4 (25)	1(10)
T2	3 (19)	1(10)
T3	3 (19)	4 (35)
T4	1 (6)	0

The biopsy sampling device is another important factor contributing to the quality of the biopsy. In 1976, Kiriyama et al. introduced the utilization of wire basket for upper urinary tract tissue biopsy [23]. Using Dormia basket, they were able to confirm the diagnosis of UTUC in 7/8 and determine grade in 5/7 cases. The advantage of endoscopic basket was further demonstrated in a study that compared the histological results of 237 forceps-obtained biopsies with 66 basket-obtained biopsies in patients with suspected UTUC [10]. UTUC was successfully diagnosed in 63% of the forceps group and 94% of the basket group ($P < 0.001$), with accurate grade determination in 80% and 93%, respectively ($P = 0.03$). In a multivariate analysis, biopsy device was the only statistically significant determinant of achieving histological diagnosis. However, when the cohort was stratified according to tumor size, the biopsy device did not predict diagnostic ability ($p = 0.2$). Indeed, in small, flat or sessile tumors, the applicability of the basket is limited and biopsy forceps are required.

In an effort to increase the size and improve quality of tissue sample achieved by forceps, a new, specially designed instrument, (BIGopsy ®, Cook Medical) has been introduced [9, 24]. This biopsy forceps appears to provide larger and deeper specimens than the ones obtained with a 3Fr forceps. However, the large (3 mm, 10 Fr) cold cup might obscure vision at the tip of the flexible ureteroscope. Even if adequate biopsy size has been achieved, the tissue sample might disintegrate during extraction, causing distortion or material loss. Gorin et al. proposed the routine use of ureteral access sheaths for a less traumatic removal of tissue fragments during ureteroscopy [8]. In a retrospective evaluation of 83 sheath-inclusive diagnostic ureteroscopies they found that 75 (90.4%) specimens were adequate for histopathologic diagnosis. In a subgroup of 34 patients who underwent NU, tumor grade on biopsy predicted the surgical tumor grade in 88%. As pointed out by the authors, a comparative study is needed to validate their results.

Despite all the technological advances, the use of small biopsy forceps for sampling a suspected renal lesion remains the only technically feasible option, in some patients. The new biopsy handling technique we describe is not intended to replace any of the various methods described herein but rather serve as an adjunct for any small volume biopsy and particularly in cases in which a biopsy can only be procured using biopsy forceps.

Our study is not without limitations. First, the retrospective nature and small sample size may restrict the applicability of our conclusions. However, as the study endpoints were strictly

Table 5 Agreement and disagreement of biopsy and nephroureterectomy specimens according to biopsy technique

	Filter paper technique *n = 16*	Standard technique *n = 11*
Grading		
Grade agreement, *n* (%)	11 (69)	7 (64)
Low grade	8	3
High grade	3	4
Upgrading, n (%)	2 (12)	1 (9)
Biopsy grade undetermined, n (%)	3 (19)	3 (27)
Staging		
Stage agreement, *n* (%)	9 (56)	3 (27)
Ta	5	2
T1	2	0
T2	2	1
Upstaging	4 (25)	0
Ta → T1	1	
Ta → T4	1	
T1 → T3	2	
Biopsy stage undetermined, n (%)	3 (19)	8 (63)

related to the pathologist interpretation and since the pathologist was blinded to the source of the biopsy we believe the results reflect the differences in the biopsy handling technique. The correlation between ureteroscopic biopsy and final surgical pathology was evaluated in a sub group of patients who underwent NU and therefore subjected to selection bias. Therefore, a randomized study is needed to confirm our results. Despite these limitations, as the proposed technique is easy to implement and does not require additional costs we believe it can only augment the currently used techniques.

Conclusions

Ureteroscopic biopsy of upper tract TCC is an essential part of the evaluation and treatment plan. The new processing technique for small, forceps biopsies described herein decreases the rate of biopsies with insufficient material and improves biopsy interpretation.

Authors' Contribution SG GUARANTOR,conception and drafting. GG: conception, revising. DM: analysis, revising. LW: analysis, revising. YE: conception, revising. RK: analysis, revising. DL: conception, and revising.**Compliance with Ethical Standards**

Conflict of Interest The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals

1) Statement of Human Rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2) Statement on the Welfare of Animals This article does not contain any studies with animals performed by any of the authors.

Informed Consent For this type of study, formal consent is not required.

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