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Urine Cytology for Detection of Bladder Carcinoma: Experience of Gezira Hospital for Renal Disease and Surgery(GHRDS)

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Abstract:

Background: Urine cytology has been and remains the standard in the initial evaluation of lower urinary tract lesions to rule out bladder cancer.

Objectives: To evaluate the significance of urine cytology as a diagnostic tool for bladder cancer and a to assess the cytological pattern in voided and washed urine.

Methodology: This was a cross-sectional descriptive hospital-based study. A total of 43 patients who were suspected to have bladder tumors based on both clinical and radiological findings. Pre-cystoscopy voided urine specimens and post-cystoscopy bladder wash specimens were collected. Three stains were used in preparation of cytological smears Hematoxylin / Eosin, Diff-Quik and May-Grünwald Giemsa, whenever possible equivalent histopathological diagnosis was obtained.

Results: This study included 86 urine cytology specimens obtained from a total of 43 cases of suspected bladder cancer. Muscle invasive cases had the higher percentage (37.2%) with more prominent features of malignancy in cytology than the earliest stages. In 30.2% of voided urine specimens were excellent, while 79.1% of bladder wash specimens had excellent quality with more cellularity and preservation of cells in cytological smears**.**

Conclusion: Urine cytology is a reliable method in the diagnosis of bladder cancer. The quality of washed urine is better than voided urine in cytological results.

Key words: urine cytology, bladder cancer, voided urine, washed urine

Introduction:

Bladder cancer (BC) represents 4.5 % of all new cancers in the USA with over 74,000 cases and it remains the 5th most common in 2015 [(1)]. Typically, it presents with hematuria, and 70 % of patients with BC initially have non-muscle invasive bladder cancer (NMIBC). NMIBC has a high chance of recurrence (60–85 %) and requires long term surveillance [(2)]. Several guidelines exist for the management of non-muscle invasive bladder cancer, and include cystoscopy and urine-based tests for initial screening and recurrence surveillance [(3-5)]. Cystoscopy is the community gold standard

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for the detection of bladder tumors, and identifies nearly all papillary and sessile tumors [6].

The gold standard for initial clinical diagnosis and surveillance of bladder cancer involves upper tract images (e.g., CT scan of the abdomen and pelvis with contrast or intravenous pyelogram) and cystoscopic examination of the bladder together with cytologic examination for malignant cells in the urine [7,8]. Cystoscopy is an uncomfortable, invasive and costly procedure [9].

Although cystoscopy is the gold standard method for the detection of bladder cancer, the false-negative results associated with cystoscopy can range from 10 to 40% [10]. With respect to carcinoma in situ (CIS), cystoscopy may miss up to 20% of the lesions because of its flat nature or resemblance to erythema from benign urologic conditions [11]. Furthermore, in a study assessing follow-up compliance of patients with bladder cancer, only 40% of patients adhered to all the tests recommended by current guidelines [12]. Consequently, the development of noninvasive urine-based assays using reliable diagnostic markers would be of tremendous benefit to both patients and healthcare systems. The urine cytology is a routine noninvasive procedure used to detect bladder cancer especially in patients with symptoms suspicious of bladder tumor. It is also used as a screening procedure to detect recurrence during the follow up of the patients who previously received treatment for bladder cancer [13,14].

Many studies identified significant variations in cytology of urine that was attributed to the method of urine collection e.g. voided, catheterized, iliac loop, washing and brushing), they found it has a significant effect on cytology results[20,21]. The aim of this study was to evaluate the significance of urine cytology as a diagnostic tool for bladder cancer and to assess the cytological pattern in voided and washed urine.

Methodology:

Setting: The study was conducted at Gezira hospital for renal disease and surgery (patient's selection and samples collection) in collaboration with the Histopathology department at medical laboratory (the site of specimens processing, examining and interpretation), Faculty of Medicine -University of Gezira.

Study design: This was prospective, cross-sectional, hospital-based study, from Jan 2016 to Dec 2016.

Study population: All Patients (43 patients) who were referred to GHRDS, suspected to have bladder tumor (Hematuria-U/S-CT) and were planned for cystoscopy with or without transurethral resection of bladder tumor (TURBT) and there was histopathology report for the resected bladder tumor postoperatively.

Methods of urine collection:

After the patient admitted to hospital and pre-operative preparations done two types of urine samples were collected in the operating room under aseptic condition:

1- Fresh voided urine sample (sample was collected pre-cystoscopy)

2-Freshbladder washing sample (cystoscopy sheath for injection and aspiration of saline)

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Both samples were submitted by the urologist. In the second sampling emptying the bladder and washing with 100 ml normal saline was done and the first 15 to 20 ml had been discarded then 25 to 45 ml was collected in a sterile container (because the first fluid in the bladder was the saline used for cystoscopy and post voided residual urine) [20].

Then specimens immediately sent to the laboratory for cytopathological evaluation (The time gap was about10 minutes from the theater to the lab)

Urine cytology:

The urine samples had been investigated for cytology in Medical laboratory - Gezira University as follow:

Rapid processing of unfixed specimens is mandatory using centrifugation. The cells were concentrated prior to transfer to glass slides, equal amounts of the sample added to two centrifuge tubes which were balanced, centrifuge with speed of 1500 rpm for 15 minutes then the cells appeared at the base of the centrifuge tube as a cell button. The supernatant was poured off carefully into a suitable disinfectant. Using a pipette, the resulting cell suspension had been removed carefully, a single small drop placed towards one end of a clean labeled glass slides and a small amount of the material had been spread to ensure that the smears were thin, thereafter smears were fixed in ethanol and then stained with Haemytoxlin and eosin (H&E). Other smears were air dried and stained with Diff-Quik and MGG stains [20].

Initial analysis was performed by histopathologist with variable experience and interest in cytopathology, whereas the review analysis was done by a senior histopathologist with wide experience in cytopathology.

Data analysis: A questionnaire was used for data collection followed by data entry using Excel (Microsoft) master sheet and then analyzed by SPSS (Statistical Package for Social Science); version 20, software for statistical analysis. Dependent and independent variables were considered to be significant if p value less than 0.05.

Ethical approval: The patients were verbally informed about the study objectives. Then the study was approved by the Ethical committee at the Faculty of Medicine, University of Gezira. Subsequently the proposal was reviewed by the division of urology research committee for feedback from urologists treating the potential participants.

Results:

The total number was 43patients but 86 samples for urine were collected for cytology (every patient has two samples voided and washed urine). The age of study population was between 19 and 80years, 11 of whom were over 60 years. We found a net predominance of malignancies in males, with a male / female ratio of 2.5:1.

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Table (1): Demographic and clinical distribution of the study population.

Age group	Number	Percentage %
• < 20	1	2.33
• 20-40	9	20.9
• 41-60	22	51.16
• >60	11	25.58
Gender		
• Male	31	72.09
• Female	12	27.91
Residence		
• Rural	12	27.91
• Urban	31	72.09
Main presenting symptom		
• Hematuria	39	90.69
• LUTS	4	9.31

LUTS= Lower urinary tract symptoms

In 30.2% of voided urine specimens were excellent, while 79.1% of bladder wash specimens had excellent quality with more cellularity and preservation of cells in cytological smears.

All patients had bladder masses seen in cystoscopy and all of them underwent TURBT, 19 patients (44.2%) had multiple foci of bladder tumors, 11 patients were in the left lateral position (25.6%), 11.6% posterior wall, 9.3% anterior wall ,4.7% trigonal and the right lateral and domal position had equal frequencies (2.3%). In 24 patients (55.8%) had solid masses and 41.9% their cystoscopy showed papillary masses (P-value 0.000). In 48.8% of the masses were medium in size, 20.9% were large ,16.3% were small and 14% were very large (P-value 0.008).

Urine cytology specimens were positive for malignancies in 29 cases (67.4%) and negative in 14 (32.6%) case. All cases positive for malignancy were transitional cell carcinoma (TCC). The urine specimens were obtained via voiding urine and bladder wash techniques for each case. Table 2 showed the quality of techniques (The quality of the three stains used in cytology were determined according to the nuclear and cytoplasmic features, and cleaning of the background of smears).

used for urine sample collections. The quality of the three stains as shown in table (2) used in cytology were determined according to the nuclear and cytoplasmic features appearance and cleaning of the background of smears.

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Table (2): Quality of different methods used for urine sample and cytological stains

Quality	Methods of urine sample		Methods of cytological stains		
	Voided Urine	Washed Urine	H/E	Diff-Quick	MGG
Excellent	30.2	79.1	11.6%	79.1%	32.6%
Very good	55.8	20.9	69.8%	18.6%	58.1%
Good	14	0	18.6%	2.3%	9.3&

There were 10 important, basic features to reaching an accurate diagnosis in cytology had been used and the results showed [22]: (Table3 and 4)

a. Very cellular smears were 69.8%, cellular 23.3% and pauci-cellular were 7 %.
b. The urothelial cells distributed in smears in single and groups represented 48.8%, fragments and single architecture had equal frequencies which were 18.6% and percentage of only group of cells were 14%.
c. The cytoplasmic features of urothelial cells in smears were as follow:
   - Variable feature of cytoplasm 27.9%, homogenous 23.3%, opaque 18.6%, bubbly and textured pale had equal frequencies which were 14%, and cytoplasmic tail had only 2.3%.
   - The large nuclear size of urothelial cells 51.2%, very large 27.9%, small 14% and enlarged nucleus were 7%.
d. The round nuclear shape was 37.2%, 30% were irregular, round to oval and oval had equal frequencies which were 16.3%.
e. The results of nucleoli features were 48.8% for absent nucleoli, 20.9 % for prominent, 18.6 % for tiny and obvious were 11.6%.
f. The nucleus to cytoplasm (N/C) ratio of urothelial cells were varied, results showed 53.5% of specimen's cells had increased N/C ratio, high and very high ratio had equal frequencies which were16.3% and 14% for low N/C ratio.
g. In 46.5% of the cases the chromatin appearance was uniform-dark, irregular- dark 23.3%, pale-uniform 16.3% and course-uniform and variable both of them were 7%.
h. The back ground of smears were haemorrhagic in 37.2% of all specimens, haemorrhagic with debris 27.9%, clear background 18.6% and inflamed in 16.3%.

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Table (3): Cellular Features in the urine cytology results

Cellularity of the smear	Frequency(patients)	Percentage%
• Pauci cellular	3	7.0
• Cellular	10	23.3
• very cellular	30	69.8
Architecture of cells in the smear		
• Single	8	18.6
• single/groups	21	48.8
• Groups	6	14.0
• Fragments	8	18.6
Other cells in the smear		
• squamous cells	10	23.3
• umbrella cells	6	14.0
• inflammatory cells	21	48.8
• glandular cells(prostatic)	3	7.0
• glandular cells(mucinous)	2	4.7
• No other cells	1	2.3

Table (4) Nuclear Features in the urine cytology results

Nuclear size in the smear	Frequency(patient)	Percentage
• Small	6	14.0
• Large	22	51.2
• very large	12	27.9
• enlarged	3	7.0
Nuclear shape in the smear		
• round	16	37.2
• round to oval	7	16.3
• oval	7	16.3
• irregular (pleomorphic)	13	30.2
Nucleoli feature in the smear		
• Absent	21	48.8
• Obvious	5	11.6
• Prominent	6	20.9
• Tiny	8	18.6
N/C ratio in the smear		

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• Very high	7	16.3
• High	7	16.3
• Increased	23	53.5
• Low	6	14
Chromatin features in the smear		
• pale, uniform	7	16.3
• Irregular, dark	10	23.3
• Course, uniform	3	7
• Variable	3	7
• uniform, dark	20	46.5

Different type of cells had been seen in cytology specimens (Figure 1), these were inflammatory cells 48.3% (A), benign squamous cells 23.3%, umbrella cells 14%, glandular prostatic cells 7% (B, C) and mucinous glandular cells 4.3% (D) and in 2.3% no other cells were seen in smears (Table1). The results of diagnosis by cytology showed 37.3% were low grade TCC (Figure 2), 30.2% were high grade TCC (Figure 3), 16.3% were reactive/inflammatory changes, 14% were normal cytology results, and only one specimen was therapy effect (table 2).

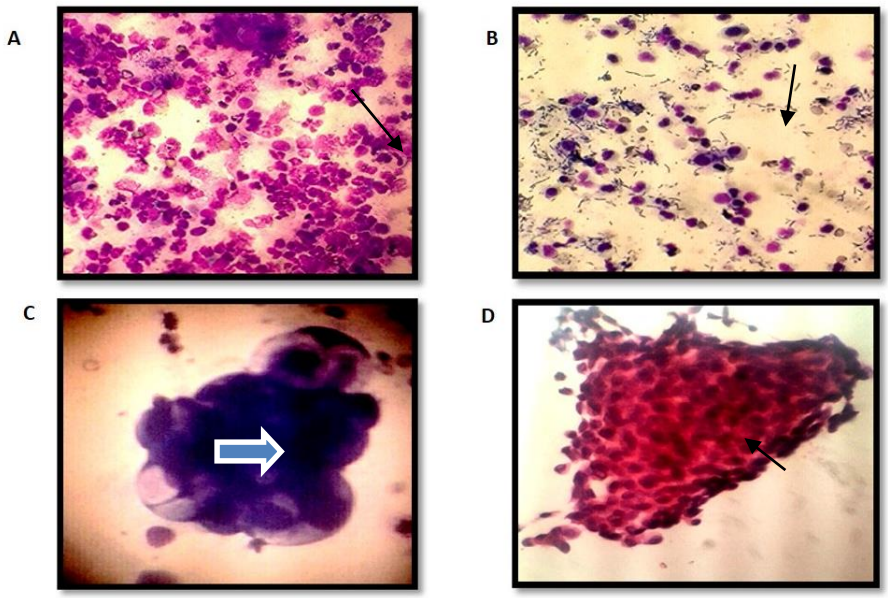

Figure (1) The copywrite of the image is kept for medical laboratory-faculty of medicine –university of Gezira (the images were taken through the microscope)
(A) A lot of benign glandular cells (prostatic) MGG (40X)
(B) A lot of benign glandular cells (prostatic) - MGG (40X)
(C) Glandular cells (prostatic) head arrow, admixed with reactive urothelial cells (blue arrow), few RBCs and debris – (Diff-Quick 40X)

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(D) A large cluster of neoplastic urothelial cells show criteria of low grade TCC H/E (40X).

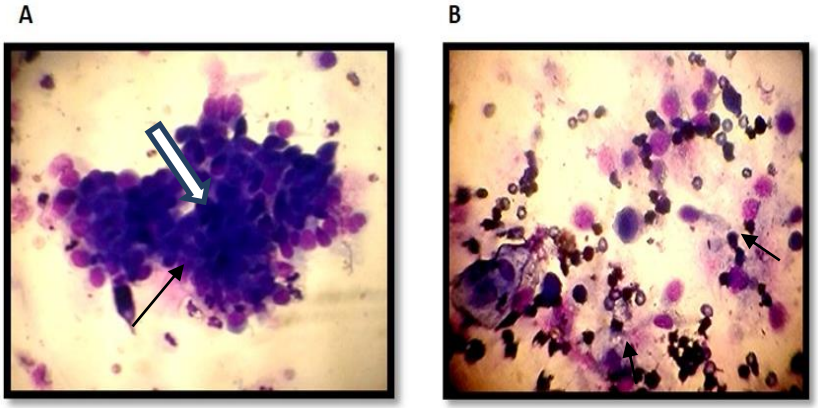

Figure (2) The copywrite of the image is kept for medical laboratory-faculty of medicine –university of Gezira (the images were taken through the microscope)
(A) A group of low grade urothelial cells (white arrow) surrounded by reactive benign cells (black arrow) Diff-Quik (40X).
(B) Low grade urothelial cells (short arrow) in a background of blood and debris, an umbrella cell is seen at left lower corner (long arrow), Diff-Quik (40X). Muscle invasive in biopsy

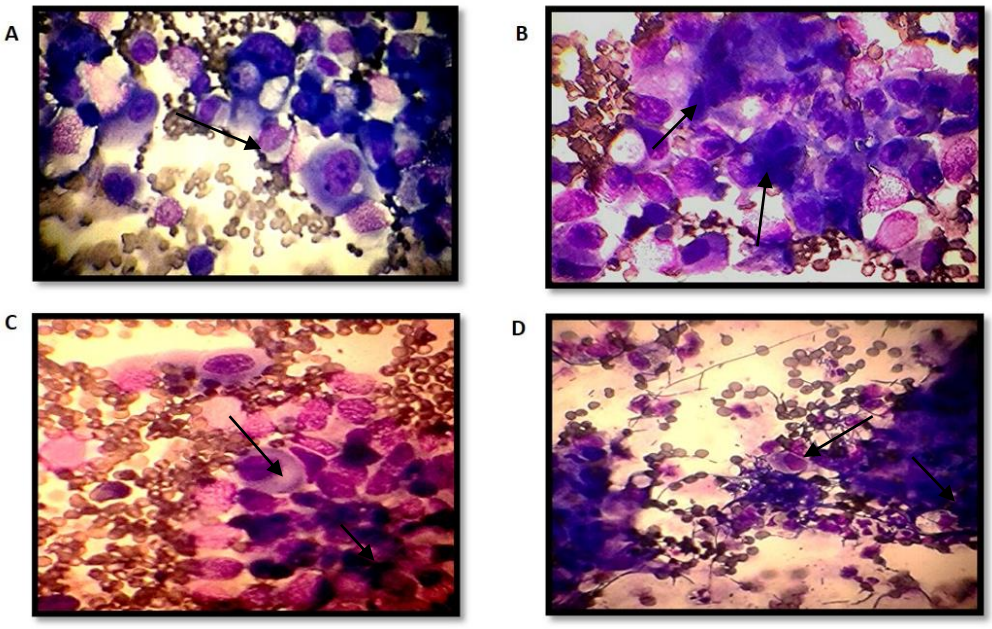

Figure (3) The copywrite of the image is kept for medical laboratory-faculty of medicine –university of Gezira (the images were taken through the microscope

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(A) High grade urothelial cells with prominent nucleoli (arrow) in haemorrhagic Background- MGG - (40X)
(B) A group of high grade urothelial cells, highly pleomorphic (arrows) MGG (40X).
(C High grade urothelial cells, pleomorphic with primitive chromatin (long arrow) in haemorrhagic background (RBCS short arrow), Diff -Quik (40X)
(D High grade urothelial cells in clusters and singly dispersed(arrows) in a background of blood and debris, Diff-Quik (40X)
Note: Muscle invasive in biopsy (We have co-related the cytology with histopathology by the result of biopsy diagnosed by other histopathologist. We have not taken an image for this biopsy).

Discussion:

Urine specimens were examined (voided and washed) from a total of 43 cases who presented to GHRDS and suspected to have bladder tumors. In the study cases, morphometric analysis of the epithelial cells on cytological smears was performed in terms of cellularity, cell architecture, nuclear size, nuclear shape, Nucleoli feature and N/C ratio (Table 3 and 4). In high grade tumors (WHO 2 and 3), nuclear pleomorphism, hyperchromasia, and high NC ratio were more marked nuclear abnormalities seen.

In this study all cases positive for malignancy were TCC. Studies carried out in USA and Europe showed that TCC is the most common type of the bladder cancer [13-14]. Other studies reported squamous cell carcinoma of the bladder as most common in the cytology results [5]. Cytological grading of urothelial carcinomas as it is an important prognostic factor for the efficacy of the treatment. Previous studies utilized cytomorphologic characteristics to grade urothelial carcinoma in urine samples reported that urine cytology was highly sensitive in detecting high grades tumor (WHO G2 and G3). On the other hand sensitivity for the detection of low-grade papillary tumors by using urine cytology is low because the cytological signs of malignancy in low-grade urothelial carcinoma are not obvious [19]. In this study cytology results showed that 37.3% were low grade TCC, 30.2% were high grade TCC, and 30.3% no malignant cells were identified. Failure of identification of low or high grade malignant cells in urine cytology might occur due to low specimen cellularity, inflammation and presence of blood in urine sample [13-14].

A most common sample from the urinary tract is spontaneous – voided urine. Bladder washing samples are also very frequent samples sent to the cytology laboratory (in Medical laboratory, Faculty of Medicine, University of Gezira). In our study the quality of samples obtained by voided urine and bladder washing were excellent in 30.2% and 79% respectively. A previous study conducted in Germany aimed to test the value of urine cytology in diagnosis of bladder cancer found that no significant difference was detected in sensitivity and specificity of voided urine and washing samples [16]. Cytologic evaluation of bladder washings necessitates caution because these specimens demonstrate artificial clustering of bladder epithelium.

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In the current study, three types of stains used in preparation of cytological smears. 79.1% of smears stained with Diff-Quik were excellent especially in preservation of cells, prominent nucleus and clean background. MGG had 32.6% excellent quality, the degeneration of cells was noticed and the nuclear features were less prominent with dirty background. H/E stain had only 11.6% good quality.

Conclusion:

Urine cytology is a reliable method in the diagnosis of bladder cancer. The quality of washed urine is better than voided urine in cytological results.

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