Urothelial Carcinoma: Is Urine Cytology an Important Diagnostic Test?

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Abstract

Introduction: Bladder carcinoma is one of the top ten malignancies in men and most common urological malignancy in both sexes. Due to its high incidence it is imperative to have a simple and reliable panel of investigations for the diagnosis of this tumor. The first line investigations usually done are urine cytology, cystoscopy and histological examination of tissue sections. Urine cytology due to its simplicity and cost effectiveness has been used effectively in the past to diagnose and follow patients with Urothelial cell carcinoma (UCC). Despite new diagnostic methods like imaging, immunohistochemistry and molecular biology techniques, urine cytology still has an important role in detecting more advanced and higher grade urothelial cancers.

Objective: The objective of this study was to find the Correlation of urine cytology with histopathology of urinary bladder carcinoma.

Methodology: The study consisted of 49 cases of Bladder neoplasms. On histopathological examination there were 47 cases of Papillary urothelial neoplasm of Low Malignant Potential (PUNLMP), Low and High grade papillary urothelial carcinoma and 2 cases were of Squamous cell carcinoma. The urine cytology slides of the same patients were examined for atypical/malignant cells and then the diagnosis was confirmed by examining the histology slides which were taken as a gold standard. The data was entered on SPSS version 19 and Correlation between the two techniques was studied.

Results: Out of 49 cases only one patient was a female with the rest being male patients. Majority of the patients were between 50-70 years age. According to the grade, most of the cases were of High Grade Urothelial cell carcinoma (57.14%). Most of the High grade cases of urothelial carcinoma ie. 24 out of 28 cases (85.7%) revealed malignant cells in urine cytology. There were 6 cases of Low Grade Urothelial cell carcinoma revealing positive urine cytology in 4 cases (66.7%) and all the 13 cases of the PUNLMP category had negative urine cytology. There were only 2 cases of Squamous cell carcinoma and there was positive urine cytology in both of the cases.

Conclusion: Urine cytology along with cystoscopy is an effective test for diagnosing urothelial cell carcinomas. It is a cost-effective, simple and a useful test. It gives a useful pre-operative opinion about the nature of bladder growth and can guide the surgeon. Due to low sensitivity of urine cytology in PUNLMP it should be combined with additional tests like immunohistochemistry and immunofluorescence along with cystoscopy.

Key words: Urinary bladder neoplasm, carcinoma, transitional cell, urine, cytology, diagnostic test

Introduction

Urothelial cell carcinoma (UCC) earlier called Transitional cell carcinoma, comprises 90% of all primary tumors of urinary bladder and it is the commonest tumor encountered by a urologist. Other important histologic types of urinary bladder cancers include squamous cell carcinoma and adenocarcinoma. In Pakistan bladder carcinoma is one of the top ten malignancies in men and most common urological malignancy in both sexes. Due to its high incidence it is imperative to have a simple and reliable panel of investigations for the diagnosis of this tumor. The first line investigations usually done are urine cytology, cystoscopy and histological examination of tissue sections. Urine cytology due to its simplicity and cost effectiveness has been used effectively in the past to diagnose and follow patients with UCC. Urine cytology is an accepted diagnostic tool in post-operative follow-up of patients with urothelial cell carcinoma. Despite new diagnostic methods like imaging, immunohistochemistry and molecular biology techniques, urine cytology still has a role to play in detecting more advanced and higher grade urothelial cancers. Urine cytology has excellent specificity with few false positive cases in high grade carcinoma. Its sensitivity is however poor especially in low grade tumors, as it is difficult to identify well differentiated cells of these tumors.
which morphologically resemble normally shed urothelial cells. Urine cytology is mostly indicated in patients with lower urinary tract symptoms like haematuria, dysuria and frequency. It is also done for follow up of patients treated for urothelial carcinoma and for screening high risk people with a history of exposure to carcinogenic substances like aniline dyes, aromatic amines and amides. The role of cytology is more important in detecting tumor recurrence where the visible tumor mass has been treated by intravesical therapy and residual tumor cannot be visualized by cystoscopy. The greatest value of urine cytology is in the follow-up evaluation of patients who received surgical or radiotherapeutic treatment for bladder carcinoma. Low sensitivity of cytology in cases of low grade tumors makes it inadequate as a screening and a diagnosing modality. However, urine cytology is often used in combination with urethrocystoscopy to detect bladder cancers in high risk population, particularly when urine analysis reveals clinically relevant hematuria. Residual tumors and high grade flat urothelial carcinoma can also be detected on urine cytology, while these lesions can be missed on cystoscopy. If urine cytology is positive then a transurethral resection of bladder tumor (TURBT) is planned and excised tissue is submitted for histopathological examination. Tissue diagnosis on H&E stain of bladder cancer is the gold standard for tumor diagnosis. The correlation of Urine cytology with histopathological diagnosis in high grade tumors highlights the importance of urine cytology in diagnosing different tumors of urinary bladder. In literature different studies suggested that the incidence of positive urine cytology is low in low grade urothelial carcinoma while it is high in high grade lesions. This study was conducted to analyze the importance of urine cytology and its role as a screening test in diagnosing bladder neoplasms, particularly urothelial tumors of various grades.

Materials and Methods

This Cross-sectional comparative study was conducted at Department of Pathology, Pakistan Institute of Medical Sciences (PIMS), Islamabad over a period of 24 months, from January 2011 to Dec 2012. The study consisted of 49 cases. There were 47 cases of Papillary urothelial neoplasm of Low Malignant Potential (PUNLMP), Low and High grade papillary urothelial carcinoma and 2 cases were of Squamous cell carcinoma. The sampling was done by convenience (non-probability) sampling. Patients of all age groups and both sexes with a clinical suspicion of bladder growth or history of bladder growth were included. Patients with reactive bladder conditions were excluded. The samples obtained were from transurethral resection of bladder tumor (TURBT) in most cases; only one case was of total cystectomy. After gross examination representative sections were submitted for histopathological examination. Processing of the tissue was carried out by automatic tissue processor, paraffin embedding, cutting, staining with Haematoxylin and Eosin was followed by mounting of slide. The urine cytology slides of the same patients were examined for atypical/malignant cells and then the diagnosis was confirmed by examining the histology slides (considered gold standard). Only those cases were selected which revealed malignancy of urinary bladder. The neoplasm was graded on basis of architecture and cytological atypia according to WHO/ISUP grading 2004. Correlation between the two techniques was made and a record was made of these findings. The data was analyzed by SPSS version 19.

Urine Sample Collection: The voided urine sample was collected in a urine container and submitted in the laboratory. Approximately 10 ml of the submitted urine was taken in a test tube. The urine was centrifuged in the centrifuge machine at a rate of 4000 rev/min for 4-5 min. The supernatant was tipped off and the smear was made with the urine sediment. The smear was then wet fixed in 98% isopropyl alcohol for 15-20 min. This was later stained with Haematoxylin and Eosin stain.

The prerequisites for collection of urine sample were as follows:

- Freshly voided urine
- 30-50 ml approximately
- Three consecutive samples in high risk cases
- First morning specimen discarded as cells undergo degeneration
- Urine samples processed within one hour of collection to avoid degeneration, bacterial overgrowth and crystal accumulation.

Histological parameters for malignancy were both architectural and cellular. Architectural atypia was papillary thickness of more than seven layers thick and fused papillae. The cellular features were pleomorphism including high nuclear cytoplasmic ratio (N/C ratio), hyperchromasia, chromatin clumping, irregular nuclear membrane and multiple nucleoli. Mitosis of more than 5/10 HPF was noted as a feature of malignancy. These parameters had to be distributed randomly throughout the thickness of epithelium. The parameters of malignant cell in urine cytology were increased cellularity and cell clusters. The cells with high N/C ratio, hyperchromasia, chromatin clumping and irregular nuclear membrane were considered malignant.

Results

This study consisted of 49 cases of Urinary bladder tumors. Only one patient was a female with the rest being male patients. Majority of patients (65.3%) were between 50-70 years age (fig.1). According to the grade, most of the cases (57.14%) were of High Grade Urothelial cell carcinoma (fig. 2). Most of the High grade cases of urothelial carcinoma ie.24 out of 28 cases (85.7%) revealed malignant cells in urine cytology with only 4 cases showing negative urine
cytology. There were 6 cases of Low Grade Urothelial cell carcinoma revealing positive urine cytology in 4 cases (66.7%) and all the 13 cases of the PUNLMP category had negative urine cytology (Table 1). There were only 2 cases of Squamous cell carcinoma and there was positive urine cytology in both of the cases. Histological and cytological features of High grade Urothelial cell carcinoma are shown in figure 3. Histological and cytological features of Squamous cell carcinoma are shown in figure 4.

Discussion

Urothelial carcinoma is the most common bladder tumor accounting for more than 90% of bladder tumors. It is the fourth most common tumor after prostate, lung and colorectal cancers making 6.4 % of all the cancers. Peak incidence is in 7th decade with male to female ratio of 3:1.1 Patients with symptoms of lower urinary tract obstruction such as frequency or haematuria are usually advised urine cytology as preliminary investigation. If urine cytology is positive for malignant cells then cystoscopy is advised, which is a very useful procedure. The surgeon can visualize areas of papillary growth or any erythematous patches within urinary bladder. However growths in bladder diverticula, or tumors masked by extensive chronic inflammation or carcinoma in situ can be missed or overlooked during cystoscopy.7 These lesions can be picked by urine cytology. However in cases of negative urine cytology, the test is repeated in high risk group and other investigations are done to reach a diagnosis. When the cytology reveals atypical cells than the patient is further investigated.

In this study, UCC was seen in male patients within age bracket of 50-70 years (65%). There was only 1 female patient, the rest of 48 patients were males. The male sex prevalence could be due to the association of UCC with patient, the rest of 48 patients were males. The male sex bracket of 50-70 years (65%). There was only 1 female in this study, UCC was seen in male patients within age incidence is in 7th decade with male to female ratio of 3:1.1. Patients with symptoms of lower urinary tract obstruction such as frequency or haematuria are usually advised urine cytology as preliminary investigation. If urine cytology is positive for malignant cells then cystoscopy is advised, which is a very useful procedure. The surgeon can visualize areas of papillary growth or any erythematous patches within urinary bladder. However growths in bladder diverticula, or tumors masked by extensive chronic inflammation or carcinoma in situ can be missed or overlooked during cystoscopy. These lesions can be picked by urine cytology. However in cases of negative urine cytology, the test is repeated in high risk group and other investigations are done to reach a diagnosis. When the cytology reveals atypical cells than the patient is further investigated. In this study, UCC was seen in male patients within age bracket of 50-70 years (65%). There was only 1 female patient, the rest of 48 patients were males. The male sex prevalence could be due to the association of UCC with smoking and driving as a profession. According to a study performed in Rawalpindi the male to female ratio was 27:1. They showed the male to female ratio to be 3.6:1. A study done in Saudi Arabia showed UCC in about 89% male patients whereas in another study done in Saudi Arabia the male to female ratio was 4.4:1. In this study UCC was predominant in older age group. Similar results were seen in another study i.e. 50 years was the predominant age.11

The low grade carcinomas have well differentiated cells and these being more cohesive, tend to have a less tendency to be shed in urine. This results in low sensitivity and specificity of urine cytology for low grade carcinoma. Features like cell clusters, hyperchromasia and nuclear membrane abnormality are helpful while evaluating atypical cells in urine cytology.12 But still the atypical cells have to be differentiated from degenerated and apoptotic cells and the cells altered by radiation. There is a considerable accepted significance of atypical cells in urine cytology.13,14 Many authorities believe that these cases should be worked up further for urothelial neoplasms as most of them turn out to be low grade UCC.15,16 In this study, we considered the atypical cases as malignant and 66.6% turned out as low grade urothelial cell carcinomas on subsequent histopathological examination. The low grade lesions usually have a history of multiple superficial recurrences in 70% to 80% of patients, with only a minority (10% to 15%) progressing to muscle invasion or metastatic disease.9 Because patients with low-grade TCC are at low risk for progression, they are monitored primarily for the development of a high grade tumor. It can be said that detection of new low-grade lesions is of secondary importance compared to the early detection of disease progression. The performance of urine cytology in this regard is much improved. Most reported sensitivities for low-grade tumors are in the range of 30–60%.17,18,19

In this study the urine cytology revealed scanty malignant cells in low grade neoplasms whereas the smears were more cellular in High grade neoplasms. Necrosis and hemorrhage was more marked in high grade lesions. Urine cytology is also an important tool in the diagnosis of upper urinary tract tumors. In these cases maximum results can be obtained by selective ureteral catheterization.20 Urine cytology can detect malignant cells even with negative cystoscopy. A positive urine cytology is to be given weightage even in cases with negative bladder biopsy. Imaging and cystoscopy should be repeated as bladder diverticular urothelial cancer or upper urinary tract cancer could be detected eventually.21 The specificity of urine cytology for High grade lesions falls around 100% and so it is a very useful test in these cases. There are some studies in which the sensitivity and specificity was 97% and 96%. Positive and negative predictive values for urine cytology were 98.5% and 92% respectively.3 However the finding of malignant cells in urine does not localize the tumor and further investigations are required which may be invasive or require radiation exposure.22 For high grade lesions, malignant cells can be picked up on urine cytology even before a cystoscopically detectable gross lesion is present.7 The sensitivity of cytology for high-grade urothelial cell carcinoma (UCC) was 90%.4 In high grade tumors the cells being dys-cohesive tend to shed more frequently in urine, resulting in a high rate of malignant cells appearance in urine. The urine cytology was 85.6% positive for malignant cells in high grade UCC in this study. The High grade lesions had a positive urine cytology establishing a correlation between the two techniques. The importance of urine cytology in diagnosing high grade lesions is highlighted by 85.6% positivity of urine cytology. Among all, 4 cases did not show malignant cells in urine cytology. The reason could be degeneration of malignant cells, low yield of cells in urine sample or the masking effects of inflammation. We also observed that the low grade carcinoma showed a significant positivity on
These patients have to be followed up more closely as they have greater chances of recurrence. However in PUNLMP negative urine cytology revealing a negative correlation between histopathology and urine cytology was seen in all the 13 cases. SCC is the second most common epithelial tumor of the bladder, accounting for 4–6.7% of vesical malignant disease in western countries. The incidence is much higher in some areas of East Africa and the Middle East, where Bilharzia is endemic and where SCC is the most common carcinoma of the bladder, as well as the most common cancer in male patients representing a major public health issue. In our study there were two cases of SCC, however these were not associated with Schistosomiasis. Both of the cases revealed positive urine cytology and muscle invasion, correlating to their aggressive behavior.

The high rate of sensitivity of urine examination indicated the great value and importance of urine cytology in evaluating HGUCC. As urine cytology is simple and quick to perform and cost effective test, it can be used in peripheral rural health centers to screen suspected patients like those with haematuria. The specimen type can also affect the interpretation of urine cytology, with voided specimens being more specific but slightly less sensitive than instrumented urine.

**Table 1: Urine Cytology in Different Grades of Urinary Bladder Carcinoma**

<table>
<thead>
<tr>
<th>Histological Grade</th>
<th>No. of cases</th>
<th>Cases showing Malignant cells in urine cytology</th>
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<tbody>
<tr>
<td>High Grade UCC</td>
<td>28 (57.14%)</td>
<td>24 (85.7%)</td>
</tr>
<tr>
<td>Low Grade UCC</td>
<td>6 (12.76%)</td>
<td>4 (66.7%)</td>
</tr>
<tr>
<td>PUNLMP</td>
<td>13 (26.53%)</td>
<td>0 (100%)</td>
</tr>
<tr>
<td>SCC</td>
<td>2 (4%)</td>
<td>2 (100%)</td>
</tr>
</tbody>
</table>

**Figure 1. Age incidence in urinary bladder carcinoma (n 49)**

**Figure 2. Prevalence of various grades of Urinary bladder carcinoma-n:49**

**Figure 3a: High grade UCC Histology (H&E 10x10)**

**Figure 3b: High grade UCC cytology (H&E 10x40)**
Figure 4a: Squamous cell carcinoma Histology (H&E 10x10)

Figure 4b: Squamous cell carcinoma Cytology (H&E 10X40)

Conclusion

Urine cytology along with cystoscopy is an effective test for diagnosing urothelial cell carcinomas. It is a cost-effective, simple and a useful test. It gives a useful pre-operative opinion about the nature of bladder growth and can guide the surgeon. However due to low sensitivity of urine cytology in PUNLMP it should be combined with additional tests like immunohistochemistry and immunofluorescence along with cystoscopy.

References