The Utility of Fluorescence in Situ Hybridization for Early Detection of Bladder Urothelial Carcinoma in Comparison with Urine Cytology

Mamdouh Roshdy¹, Olfat Hammam², Ahmed Abdelsalam¹, Hani Nour¹, Hosam El Ganzori¹, Samir Ghobashy¹ and Ahmed Kamel¹

Urology¹ and Pathology² Departments, Theodor bilharz Research Institute, Scientific Research Academy, Egypt. (hossam_elganzouri@yahoo.com); (totoali1@hotmail.com)

Abstract: UroVysion is a multitarget multicolor fluorescent in situ hybridization (FISH) probe set combining 4 probes in single probe that detects aneuploidy of chromosomes 3, 7, and 17 and loss of the 9p21 locus in exfoliated urine cells, our study investigated the role of UroVysion in predicting tumor recurrence in patients with free cystoscopy and –ve cytology and detecting new cases of urothelial carcinoma of the bladder in patients complaining of hematuria with high risk of malignancy. Material and Methods: our study conducted on 46 patients, group A: 30 patients with history of superficial urothelial carcinoma and 16 patients complaining of gross heamaturia subdivided after cystoscopy into group B) 8 patients with 1ry urothelial carcinoma and group C) 8 patients with cystitis. All patients were subjected to voided urine cytology and UroVysion before cystoscopy ± biopsy. Follow up of group A and one case of group C with +ve UroVysion was done for 18 months. Results: The mean age of patients was 50.5 years. In group A, 15 (50%) patients were UroVysion –ve and 15 patients were UroVysion+ve and all patients are negative for malignancy by cytology and first cystoscopy was free. Recurrence were diagnosed in 11 patients (36.6%) with median time to recurrence was 4 months. Anticipatory +ve UroVysion preceded recurrence in 81.8% and NPV was 86.6%. There was statistical significance p<0.01 difference in UroVysion results between low and high grades. For group B the sensitivity of UroVysion was equal to cytology (75%), Group C only one patient was +ve FISH and developed low grade Ta TCC after 12 months. Bilharzisis had no influence on the predictive value of UroVysion. Conclusion: Our data suggested that UroVysion help to predict recurrence of uothelial cell carcinoma of the bladder and may change the surveillance protocols especially patients with history of low grade tumors with –ve UroVysion to be with expanded intervals.

Key words: Urothelial cancer, UroVysion, follow up, bilharziasis.

1-Introduction

Worldwide, bladder cancer is the 9th most common cancer (Feraly et al., 2004). In Egypt, carcinoma of the bladder is the foremost oncologic problem. At the National Cancer Institute (NCI), Cairo, it constitutes 30.3% of all cancers, 40.6% of male cancers, and 14.3% of female cancers (El-Bolkainy, 2000).

Two main histological types of bladder cancer are identified: the transitional cell carcinomas (TCC), related to cigarette smoking and most prevalent in Western and industrialized countries, and the squamous cell carcinomas (SqCC), which are more frequently seen in some Middle Eastern and African countries, where urinary schistosomiasis is an endemic disease (Sengupta et al., 2004).

Transitional cell carcinoma comprises 90% of primary tumors of the bladder and 30-40% of carcinomas associated with schistosomiasis. In past decades, increased incidence of transitional cell carcinoma associated with schistosomiasis has been reported (Khalil et al., 2003).


The WHO recommendation to move away from the traditional grading system (1 to 3, from low grade to high grade) is now accepted by many urologists and pathologists, and recommended that malignant tumors should be classified as low grade or high grade, regardless of invasion status. The variance in biologic behavior for low-grade versus high-grade lesions correlates with the known dual molecular lines of genetic development for these two pathways supports the concept that high-grade and low-grade cancers may be considered as essentially different diseases (Droller, 2005).

The gold standard for diagnosis and surveillance of urothelial carcinoma has traditionally been cystoscopy and urine cytology, however both have limitations. Although urine cytology is excellent for
detecting high-grade urothelial carcinoma (sensitivity and specificity 75%), it has a low sensitivity (20–60%) for detecting low-grade tumors (Skacel et al., 2003).

The low sensitivity of urine cytology, the invasiveness of cystoscopy and its limited usefulness in detecting flat and inaccessible lesions have prompted increased demand for newer, more sensitive and non-invasive tests for detection of urothelial carcinoma. This has led to the development of new sophisticated molecular techniques that have a higher sensitivity and specificity for urothelial carcinoma detection (Halling et al., 2002).

One such technique is the now commercially available multicolor, multitarget, UroVysion fluorescence in situ hybridization (UroVysion FISH) assay that was created by Vysis Incorporated (Vysis–Abbot Laboratories, Downers Grove, IL, USA) (Halling et al., 2003). UroVysion FISH is the first molecular test that uses DNA (Deoxyribonucleic acid) probes to identify the most common urothelial carcinoma related chromosomal abnormalities in urine. The Food and Drug Administration (FDA) initially approved its use in 2001 as a surveillance tool for patients with history of urothelial carcinoma. However, this was later in 2005 extended to include its use as a screening tool in patients with hematuria and risk factors for urothelial carcinoma (www.fda.gov).

UroVysion FISH has a reported sensitivity of 73–92%, a specificity of 89–96% for urothelial carcinoma detection and several studies have confirmed its usefulness in the diagnosis and surveillance of these tumors (Glas et al., 2003).

The test is designed to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus at chromosome number 9 in malignant urothelial cells that are shed in the urine of persons with urothelial carcinoma. These represent some of the most common chromosomal abnormalities in urothelial carcinoma. Polysomy of chromosomes 3, 7 and 17 are associated with high-grade urothelial carcinoma, while deletions of chromosome 9p21 (where the p16-INK4A tumor suppressor gene resides) are seen in up to 60% of superficial low grade, papillary tumors. (Skacel et al., 2003). In the trial that led to FDA approval of UroVysion, Sarosdy and coworkers reported 36 patients with a negative cystoscopic examination but a positive FISH result. With continued longitudinal follow-up, 15 (41.7%) of these cases were found to have biopsy-proven tumor recurrence with time-to-tumor diagnosis of 3–16 months (mean 6.0 months). Conversely, among 68 patients who had a negative cystoscopy and a negative FISH result, only 13 (19.1%) had a biopsy proven recurrence at 3–19 months (mean 11.2 months). The patients with a positive FISH result but negative cystoscopy were referred to as anticipatory positive cases. A Kaplan-Meier curve revealed that the time-to-recurrence was significantly less (p = 0.014) for the patients with anticipatory positive FISH results compared with those with negative FISH results. (Sarosdy et al., 2002).

**Aim of the work:** our study investigated the role of UroVysion in predicting tumor recurrence in patients with free cystoscopy and –ve cystoscopy and detecting new cases of urothelial carcinoma of the bladder in patients complaining of hematuria with high risk of malignancy.

**2-Patients and Methods:**

A total of 46 patients were enrolled in this prospective study including 42 males and 4 females, their ages ranges from 41-80 years (mean age 50.5 years), all patients were admitted to the Urology department of Theodor Bilharz Research Institute (TBRI).

The study protocol was approved by the Ethics committee of Theodor Bilharz Research Institute (TBRI) according to the institutional committee for the protection of human subjects and adopted by the 18th world medical assembly, Helsinki, Finland.

The patients then were categorized into 3 groups:

Group A: 30 patients with past history of superficial TCC of the bladder with no apparent lesions with imaging. The inclusion criteria for group A are that all patients had no evidence of recurrence in the last 3 months prior to enrolment in the study and the first cystoscopy and cytology were negative. Group B: 8 patients with primary bladder urothelial carcinoma diagnosed by imaging out of the 16 patients complaining of hematuria, and Group C are the rest 8 patients diagnosed as cystitis associated with Schistosomiasis and consider as negative control and no apparent masses were found at cystoscopy, and cold cup biopsies were taken from any suspicious areas (2 with gross and 6 with microscopic hematuria), irrigative micturition symptoms and with negative imaging.

All patients were subjected to the following: Detailed history taking and a full clinical examination, routine laboratory investigations, urine analysis, complete blood count (CBC), liver and kidney function tests, Abdomino-pelvic ultrasonography (U/S), Intravenous urography (IVU), Computed tomography scan (CT) of the abdomen and pelvis in selected cases, voided urine cytology, voided urine FISH analysis by multicolor uroVysion kit.

**Follow up protocol:**

Group A: Cystoscopy every 3 months for 18 months follow up period, and TURT was done for
recurrent cases. Group C: Cystoscopy every 6 months for 18 months follow up and only one patient in group c who's FISH test was positive followed up with cystoscopy every 6 months

**Histopathological techniques:**

All TUR and cold-cup specimens were fixed for 24 hours in 10% neutral buffered formalin solution, and then processed for preparing paraffin blocks. The paraffin wax sections were cut at 5 microns.

All slides were treated with 3-amino-propyl-triethoxysilane (3-APTES/SIGMA-A-3648); these slides were used instead of the ordinary albuminized slides to minimize staining artifacts and for better fixation of the sections on the slides. Hematoxylin and eosin stains was done for routine histopathological examination and diagnosis and the presence of bilharzial infestation. Tumor grading and staging system were done according to the world health organization (WHO) (Eble et al., 2004).

**Cytopathological diagnosis:**

The urine samples were collected into clean tubes for cytopathologic diagnosis. Part of the collected samples was centrifuged at a rate of 1200–1500 rpm for 15 minutes using Shandon Cytospin (ThermoFisher Scientific, Waltham, Massachusetts). The sediment was smeared on slides that were pretreated with 3-APTES (3-amino-propyl-triethoxy saline, Sigma-Aldrich Ireland Ltd, Dublin, Ireland). Slides were fixed immediately in 95% ethanol for 24 hours and then stained with hematoxylin and eosin. And unstained slides kept for FISH stain. Urinary specimens submitted for UroVysion FISH were transported in an acceptable manner (fixed in PreservCyt [Hologic, Inc., Bedford, Mass] or Saccomanno fixative) with a minimum volume of 33 mL.

**Fluorescence In Situ Hybridization**

Urine smears from all different studied bladder lesions were stained for UroVysion using Vysis Bladder cancer Kit. And UroVysion FISH was performed as rec-ommended by the probe manufacturer (Abbott Molecular, Inc.) using VP2000 and HYBrite instruments (Abbott Molecular, Inc.) for pre-hybridization and hybridization steps, respectively. Slides were stored in the dark at 20C until screening. ProbeChek UroVysion control slides were processed and evaluated along with patient slides to monitor UroVysion FISH labeling and reproducibility.

**UroVysion Interpretation By FISH In Bladder Lesions:**

UroVysion consists of a mixture of 4 different DNA probes, each with a different fluorophor. Three centromere probes for chromosomes 3, 7, and 17 are employed (marked with red, green, and aqua fluorophors), as well as a probe for the 9p21 locus, linked to a gold fluorophor. These probes allow for the detection of aneuploidy of chromosomes 3, 7, and 17, detectable as extra (i.e., >2) signals in the nucleus for each of the markers.

Interpretation of FISH slides. Slides were evaluated by Pathologist blinded to patient clinical history and pathological findings. Slides were assessed by y scanning for cytologically atypical cells and determining the number of CEP3, CEP7, CEP21 and 9p21 signals in these cells. Atypical cytological features included patchy and lighter nuclear DAPI (4,6-diamidino-2-phenylindole) staining, nuclear enlargement and irregular nuclear contour as previously described. Gains involving 2 or more of these 3 chromosomes are referred to as polysomy. The criteria used for a "positive" UroVysion test is the detection of 4 or more cells that have gains in 2 or more of chromosomes 3, 7, and 17 in the same cell, or the identification of 12 or more cells that show deletion of the 9p21 locus or 10 or more demonstrated trisomy or greater than 20% demonstrated 9p21 homozygous deletion.

If a positive diagnosis was rendered, 100 consecutive urothelial cells (non-inflammatory or non-squamous cells were considered urothelial) were analyzed to determine the percent of urothelial cells in urine that demonstrated an abnormal FISH pattern. All patients with a positive FISH result in this study were found to have polysomy. There were no patient samples that showed trisomy or homozygous 9p21 deletion.

**Statistical analysis:**

Contingency table analysis was used to calculate the association between grading, pT stage, and FISH results. For the analysis of sensitivity, specificity, and positive and negative predictive values of multiprobe FISH, only the cases were included in which cytopathologic grade also was available for comparison. Estimation of recurrence-free survival was performed by a log-rank test.

**3- Results**

A total of 46 patients were enrolled in this prospective study including 42 males and 4 females, their ages range from 41-80 years (mean age 50.5 yr). 30 patients had past history of superficial TCC of the bladder and 8 patients were newly diagnosed as bladder mass by imaging and 8 patients two of them presented by total gross hematuria and 6 patients with microscopic hematuria and all with negative imaging. We performed for all patients voided urine cytology, voided urine FISH analysis by multicolor uroVysion kit, and cystoscopy.

**The patients were stratified into 3 groups:**

For group A: The first follow up cystoscopy was free from tumors and repeated follow up
cystoscopies were done every 3 months for 18 months and TURT was done for recurrent cases.

For group B: TURT was done.

For group C: Cold cup biopsies were taken from suspicious areas and only one case with FISH +ve was followed up by cystoscopy every 6 months.

**Group A:**

We have found that 15 patients (50%) were FISH +ve and 15 patients (50%) were FISH –ve. All patients were negative for malignancy by cytology and first cystoscopy was free. Recurrence were diagnosed in 11 patients (36.6%) with median time to recurrence was 4 months, while 19 patients (63.3%) had no recurrence in the 18 months follow up period. Positive UroVysion FISH preceded the diagnosis of tumor recurrence in 9/11 cases (81.8%). Only 2 patients with negative UroVysion test developed a recurrent tumor. Therefore the negative predictive value (NPV) for a negative UroVysion test is (86.6%). Negative UroVysion FISH results was found in 13/19 cases (68.4%) that did not show recurrence for 18 months follow up (Figure 1).

*By stratification of group A by histopathological tumor grade:

The highest tumor grade before enrollment in our study was low grade in 24 cases (80%) and high grade in 6 cases (20%). Nineteen patients diagnosed as low grade cases (79%) had no recurrence, 13 of them were FISH –ve (68.4%) and 6 were FISH +ve (31.6%). Five of the low grade cases developed recurrence, positive UroVysion test preceded the diagnosis of recurrence in 3/5 cases (60%). For the patients with low grade tumors sensitivity of FISH was 60%, specificity 68.4%, PPV 33.3% and NPV 86.6% (Table 1).

All cases of high grade TCC were preceded by positive UroVysion test before the diagnosis of recurrent tumors with sensitivity and PPV 100%. Anticepatory positive FISH preceeded the diagnosis of recurrence in 3/5 low grade tumors and 6/6 high grade tumor (Table 1).

*By stratification of group A by histopathological stage:

In our study we found that: Ten cases (33.4%) were Ta and 20 cases (66.6%) were T1. 9 cases of Ta (90%) had no recurrence, 5 cases of them were FISH –ve (55.5%) and 4 cases were FISH +ve (44.5%). 1 case only of Ta (10%) had recurrence and preceded by +ve FISH test. For patient with Ta tumors sensitivity of FISH was 100%, specificity 55.5%, PPV 20%, NPV 100% (Table 2).

Ten cases of T1 (50%) had recurrence, 8 cases were FISH +ve (80%) and 2 cases were FISH –ve (20%), 10 cases of T1 (50%) had no recurrence, 8 cases were FISH –ve (80%) and 2 cases were FISH +ve (20%). For T1 sensitivity, specificity PPV and NPV, of FISH were 80 % (Table 3)(Figures 2,3,4,5,6,7).

**Group B:**

In our study, there were 8 newly diagnosed cases of TCC of the bladder:

Six cases were T1 (75%), and 2 were T2 (25%), 6 cases were high grade and 2 cases were low grade. The 2 cases of the low grade tumors were FISH –ve and cytology –ve for malignancy (25%), and the other 6 patients with high grade tumors were FISH +ve and cytology +ve for malignancy (75%). Sensitivity of FISH was equal to cytology (75%).

**Group C:**

Eight cases of cystitis presented by either gross hematuria (25%) or microscopic hematuria (75%), all cases had cytology –ve for malignancy but one case was FISH +ve for malignancy (75%). Sensitivity of FISH was equal to cytology (75%).

**FISH in patients with bilharziasis:**

In our study bilharziasis was diagnosed in 13 patients (28.2%). Six patients had past history of superficial TCC of the bladder, five patients presented by primary TCC of the bladder and two patients with bilharzial cystitis. Positive UroVysion FISH test was found in (50%) of patients in either groups, who developed and not developed recurrence. Two out of five cases with primary tumors were –ve UroVysion FISH test and all were low grade tumors.

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**Table 1:** showing UroVysion FISH of follow up TCC (Group A) cases verses histopathological grades

<table>
<thead>
<tr>
<th>Histopathological grades</th>
<th>Negative FISH cases</th>
<th>Positive FISH cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Low grade (n=24)</td>
<td>15</td>
<td>62.5</td>
</tr>
<tr>
<td>High grade (n= 6)</td>
<td>0</td>
<td>00*</td>
</tr>
</tbody>
</table>

*P<0.01 compared to low grade.

**Table 2:** showing UroVysion FISH of follow up TCC (Group A) cases verses histopathological stages

<table>
<thead>
<tr>
<th>Histopathological stages</th>
<th>Negative FISH cases</th>
<th>Positive FISH cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Ta (n=10)</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>T1 (n=20)</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

No significant difference between group
### Table 3: Sensitivity, specificity, PPV, NPV for UroVysion FISH According to grades and stages of follow up cases of TCC

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade (n=24)</td>
<td>60%</td>
<td>68.4%</td>
<td>33.3%</td>
<td>86.6%</td>
</tr>
<tr>
<td>High grade (n=6)</td>
<td>100%</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Ta (n=10)</td>
<td>100%</td>
<td>55.51%</td>
<td>20%</td>
<td>100%</td>
</tr>
<tr>
<td>T1 (n=20)</td>
<td>80%</td>
<td>80%</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>UroVysion FISH Follow up cases (n=30)</td>
<td>81.8%</td>
<td>68.4%</td>
<td>60%</td>
<td>86.6%</td>
</tr>
</tbody>
</table>

Fig. 1: A) Urine smear from a control case showing normal urothelial cells (H&E,x100) Fig. B): Urine smear from a cystitis case showing urothelial cells with moderate number of acute and chronic inflammatory cells and squamous metaplastic cells (H&E,x100). Fig. C): Urine smear from a low grade TCC case showing sheets of malignant urothelial cells with moderate anaplasia (H&E,x200). Fig. D): Urine smear from a high grade TCC case showing clusters of malignant urothelial cells with high anaplasia (H&E,x200).

Fig. 2: Urine smear from a control case showing nucleus of urothelial cells with negative FISH with normal signals of red, green, aqua and gold of chromosomes 3, 7, 17, and loss of the 9p21 locus (two copies for each) (FISH UroVysion,x1000).

Fig. 3: Urine smear from a cystitis with hematuria case showing nucleus of urothelial cell with negative FISH with normal signals of red, green, aqua and gold of chromosomes 3, 7, 17, and loss of the 9p21 locus (two copies for each) (FISH UroVysion,x1000).
Fig. 4: Urine smear from a low grade, non muscle invasive urothelial carcinoma case showing nucleus of urothelial cells with negative FISH with normal signals of red, green, aqua and gold of chromosomes 3, 7, 17, and of the 9p21 locus (two copies for each) (FISH UroVysion, x1000).

Fig. 5: Urine smear from a low grade, non muscle invasive urothelial carcinoma case showing nucleus of urothelial cells showing positive FISH with many signals of red, green, aqua and gold of chromosomes 3, 7, 17, and loss of the 9p21 locus (more than two copies for each) (FISH UroVysion, x1000).

Fig. 6: Urine smear from a high grade, muscle invasive urothelial carcinoma case showing nucleus of urothelial cells with positive FISH with many signals of red, green, aqua and gold of chromosomes 3, 7, 17, and of the 9p21 locus (more than two copies for each) (FISH UroVysion, x1000).

Fig. 7: Urine smear from a high grade, muscle invasive urothelial carcinoma case showing nucleus of urothelial cells with positive FISH with many signals of red, green, aqua and gold of chromosomes 3, 7, 17, and of the 9p21 locus (more than two copies for each) (FISH UroVysion, x1000).

4- Discussion:
Non-muscle-invasive urothelial cell carcinoma (NMIUCC) is associated with a high rate of disease recurrence (60%-85%) and progression to high-grade lesions (10%-30%), and therefore these patients must be monitored regularly (Kirkali et al., 2005).

The gold standard for NMIUCC surveillance is cystoscopy in conjunction with urine cytology (Babjuk et al., 2008). White-light cystoscopy remains the primary mode of diagnosing and monitoring bladder cancer, but the procedure is expensive, invasive, and causes patient discomfort and anxiety (Messing et al., 2000). Although this technique has a specificity of > 90%, carcinoma in situ (CIS) and flat lesions, as well as upper urinary tract lesions, may be missed (Witjes et al., 2007), (Shaw et al., 2008).

Cytology is noninvasive and offers high specificity, although it has low sensitivity in the diagnosis of low grade papillary tumors. For these reasons, multiple noninvasive assays for the detection of non invasive muscle urothelial cell carcinoma recurrence have been developed (Van Tilborg et al., 2009). The objective of the current study was to determine the clinical value of the FISH technique in 3 groups of unselected cohort of patients: Group A: Thirty patients had past history of superficial TCC of the bladder. Group B: Eight patients with newly diagnosed TCC of the bladder. Group C: Eight patients with cystitis associated with schistosomiasis.

In group A we have assessed the predictive value of FISH technique for monitoring patients on surveillance protocol for non muscle invasive urothelial carcinoma of the bladder and we found that the UroVysion FISH assay is a useful tool for predicting patient’s risk for tumor recurrence. Recurrence were diagnosed in 11 patients (36.6%) with median time to recurrence was 4 months, while
19 patients (63.3%) had no recurrence in the 18 months follow up period. Positive UroVysion FISH (i.e.; anticipatory positive) preceded the diagnosis of tumor recurrence in 9/11 cases (81.8%). Only 2 patients with negative UroVysion test developed a recurrent tumor. Therefore the negative predictive value (NPV) for a negative UroVysion test was (86.6%). Recurrent tumors were found in 60 % of the patients who had positive UroVysion test compared with 13.3% of the patients with negative test. These results were comparable to the study conducted by Gofrit et al., 2007 in which it was answering the same question about the predictive value of UroVysion FISH in surveillance of patients with NMIUC of the bladder and they stated that positive UroVysion test (ie; anticipatory positive) preceded the diagnosis of tumor recurrence in 18/21 cases (86%), including all cases of high-grade recurrence, which was comparable to our study that stated anticipatory positive FISH in 9/11 cases (81.8%). In Gofrit et al., 2007 study recurrent tumors were found in 45% of the patients who had positive UroVysion test compared with 12.5% of the patients with negative test, they also stated that the 3 patients with negative FISH who developed recurrence all were low grade tumors and the NPV was 87.5% (Gofrit et al., 2007). In other three studies addressing this question, recurrence rates in patients with positive UroVysion assay were 43.8% in the study of Maffeizzini et al., 2010, a 62.5 % in the study of Kurien et al., 2007 and 36% in the study of Zellweger et al., 2006.

We have found in our study that 19 patients diagnosed as low grade cases (79%) had no recurrence, 13 of them were FISH –ve (68.4%) and 6 were FISH +ve (31.6%). Five of the low grade cases developed recurrence, positive UroVysion test proceed the diagnosis of recurrence in 3/5 cases (60%). For the patients with low grade tumors sensitivity of FISH was 60%, specificity 68.4%, PPV 33.3% and NPV 86.6%. On the other hand all cases of high grade TCC were preceded by positive UroVysion test before the diagnosis of recurrent tumors with sensitivity and PPV 100%.

These results were comparable to a great extent to what stated by Gofrit et al., 2007 that all high grade tumors that recurred were preceded by positive FISH with sensitivity and PPV 100 %. (Gofrit et al., 2007). One clinical implication is that high-risk patients should benefit from attentive follow-up, whereas low-risk and negative patients may be equally safe with less frequent surveillance (Maffeizzini et al., 2010). Gofrit et al., 2007 concluded that if a patient with a history of low-grade bladder cancer has a negative UroVysion assay, this has two meanings: There is a very low likelihood that the patient is currently inflicted by a urothelial carcinoma and there are no transformed cells, the precursors of urothelial carcinoma in the system. Therefore, this patient has a very low risk for tumor recurrence in the coming year. Taking into account the benign natural history of low grade tumors, cystoscopy may be omitted and next follow-up visit may be scheduled to 12 months. Patients with positive UroVysion should be reassured that this does not mean an inevitable tumor recurrence, but a need for further surveillance. Patients with history of high-grade tumor and a negative UroVysion test still require a survey every 3 months, taking into account the lethal potential of these tumors. In these patients the UroVysion assay is a useful adjunct to cystoscopy and cytology. (Gofrit et al., 2007). Studying the effect of histopathological tumor stage on the predictive value of FISH for tumor recurrence:

In our study we found that: Ten cases (33.4%) were Ta and 20 cases (66.6%) were T1. Nine cases of Ta (90%) had no recurrence, 5 cases of them were FISH –ve (55.5%) and 4 cases were FISH +ve (44.5%). One case only of Ta (10%) had recurrence and preceded by +ve FISH test. For patients with Ta tumors sensitivity of FISH was 100 %, specificity 55.5%, PPV 20 %, NPV 100 %. Ten cases of T1 (50%) had recurrence, 8 cases were FISH +ve (80%) and 2 cases were FISH – ve (20%). Ten cases of T1 (50%) had no recurrence, 8 cases were FISH –ve (80%) and 2 cases were FISH +ve (20%). For patients with T1 tumors sensitivity, specificity, PPV and NPV of FISH were 80 %.

The sensitivity and NPV for Ta patients were very high (100%) because there was only one patient with history of Ta tumor who had recurrence and was preceded by +ve FISH. However, it is difficult to draw any significant conclusions about the NPV of the test on the basis of such a limited number of recurrent tumours. The PPV was very low (20%) in patients with history of Ta tumors because there were 4 false positive FISH tests. This hypothesis was proposed by Gofrit et al., 2007 that +ve FISH test does not mark the presence of a tumor but the presence of genetically altered or transformed cells in the urine. Not every transformed cell will eventually become a clinical tumor. Genetically altered or transformed cells may either undergo apoptosis with or without assistance of the host defense mechanisms or they may become dormant for various periods of time. Therefore, positive UroVysion test does not necessarily indicate a tumor in the urinary system or a definite risk for tumor recurrence. Rather, positive FISH suggest that tumor precursors that have the potential to become future tumors are present. (Gofrit et al., 2007). UroVysion in patients with history of T1 tumors were significantly predictive for recurrence.
with PPV and NPV were equal (80 %), as there were 2 false positive and 2 false negative Urovysion tests.

**Conclusion:**

Our data suggested that UroVysion help to predict recurrence of uothelial cell carcinoma of the bladder and may change the surveillance protocols especially od patients with history of low grade tumors with –ve UroVysion to be with expanded intervals.

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