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An Evaluation Study to Correlate the Findings of Urinary Liquid Based Cytology, Cystoscopic and Histopathology in Cases of Non-Muscle Invasive Urinary Bladder Carcinoma

Navjot Kaur[1], Ramesh Kumar Kundal[2], Harjinder Singh[3], Javia Singh Raina[1]

[1]Junior Resident, Department of Pathology, Government Medical College, Patiala, India.
[2]Professor & Head, Department of Pathology, Government Medical College, Patiala, India.
[3]Professor & Head, Department of Urology, Government Medical College, Patiala, India.

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ABSTRACT

Background: Urothelial Carcinoma of bladder is a heterogeneous group of tumors with different malignant potential and natural history. A cystoscopic biopsy is the most accurate technique in diagnosing bladder tumors. Exfoliative urinary cytology is the simple, noninvasive, and cost-effective procedure for screening as well as for monitoring known cases of urothelial Carcinoma. In 1990s, Liquid-based cytology was developed as an alternative to conventional cytological preparations to overcome the high false-negative rates of traditional cytology of urine. This study aims to evaluate the accuracy of urinary liquid-based cytology in diagnosing nonmuscle invasive urinary bladder carcinoma in new and follow up cases and to correlate the findings of urinary liquid-based cytology, cystoscopy and histopathology. **Methods:** This study was a prospective observational study conducted in the Department of Pathology, Government medical college, Patiala, in the year 2018-2019. The patients were divided into 2 groups. Group A consisting of 25 newly diagnosed cases of Nonmuscle invasive carcinoma of the urinary bladder and Group B, consisting of 25 follow up cases of Non-Muscle Invasive carcinoma of the bladder. Patients age between 18 to 85 years and both genders were included in this study. Cystoscopy was done in all patients for evaluation and Barbotage urine sample for LBC was obtained. All patients underwent TURBT of bladder growth and the specimen was sent for histopathology. The findings of LBC, cystoscopy and histopathology were recorded and analysed and correlated statistically. **Results:** The sensitivity of LBC in Group A patients was 52% and in Group B was 72%. The overall sensitivity calculated was 62%. The sensitivity of cystoscopy in Group a patients was 80% and in Group B was 88%. The overall sensitivity calculated was 84%. The statistical correlation between LBC and histopathology was significant i.e. Group A, p valve = 0.023 and Group B, p valve = 0.010). The statistical relationship between cystoscopy and histopathology was significant i.e Group A, p valve = 0.006 and Group B, p valve = 0.037. **Conclusion:** Barbotage specimens and exfoliative urinary cytology should be performed to complement cystoscopy. LBC has better sensitivity than conventional urine cytology as there occurs better cell preservation. The sensitivity of urinary cytology increases with tumor grade. Despite of high sensitivity of urinary cytology and cystoscopic examination of bladder mass, histopathological diagnosis remains essential because it allows definitive diagnosis, the grade of the tumor, assessment of the degree of differentiation and Tumour staging.

Keywords: Cystoscopy, Urinary Bladder Carcinoma.

INTRODUCTION

Urothelial Carcinoma of bladder is a heterogeneous group of tumors with varying malignant potential and natural history.[1] Histologically, 90% of bladder cancers are of urothelial origin, 5% are squamous cell carcinomas, and less than 2% are adenocarcinoma or other variants. At the initial presentation, 80% of urothelial tumors are non–muscle invasive. There are multiple growth patterns of urothelial cancer, including flat Carcinoma in situ (CIS), papillary tumors that can be low or high grade, and sessile tumors with a solid growth pattern.[2]

Cystoscopic biopsy has predictive value and therefore, it is the most accurate technique in diagnosing bladder tumors.[3] Flexible office cystoscopy is as reliable as rigid endoscopy and has excellent sensitivity and specificity for papillary tumors but is relatively poor for CIS. Other new cystoscopy techniques are Photodynamic diagnosis/blue-light/white light cystoscopy and narrow-band imaging.[4]

Exfoliative urinary cytology is the simple, noninvasive, and cost-effective procedure for screening as well as for monitoring known cases of urothelial Carcinoma.[5] Overall, the sensitivity and specificity of cytology in detecting bladder cancer are 40% to 62% and 94% to 100%, respectively. Detection rates are high for tumors with high grade and stage as well as CIS.[6,7] However, Poorly differentiated tumors and well-differentiated tumors have up to 20% and 80% false-negative detection rate, respectively.[2]

In 1990s, Liquid-based cytology was developed as an alternative to conventional cytological

Name & Address of Corresponding Author
Dr. Harjinder Singh
Prof and HOD, Urology,
Government Medical College,
Patiala,
India.

preparations to overcome the high false-negative rates of conventional urine cytology.[8] LBC has several advantages such as automated and standardized processing techniques that produce a uniformly distributed and cell-enriched slide, less obscuring elements in the background and the thin-layered cells require less time to make a diagnosis, the residual specimen can be used for ancillary techniques, such as immuno-cytochemistry and molecular studies.[9]

As there are limited studies on the diagnostic accuracy of Liquid-based cytology in bladder carcinomas especially in our set up we conducted a study at Government Medical College, Patiala to evaluate the accuracy of urinary Liquid-based cytology in diagnosing nonmuscle invasive urinary bladder carcinoma in new and follow up cases and to correlate the findings of urinary Liquid-based cytology, cystoscopy and histopathology.

MATERIALS AND METHODS

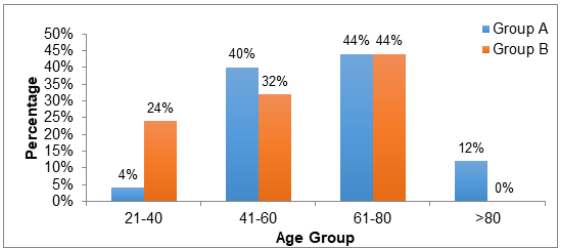
This study was a prospective observational study conducted in the Department of Pathology, Government medical college, Patiala, between 2018-2019. After thorough examination and investigation, the patients were divided into 2 groups. Group A consisting of 25 newly diagnosed cases of Nonmuscle Invasive Carcinoma of the urinary bladder and Group B consisting of 25 follow up cases of Non-Muscle Invasive carcinoma of the bladder. Patients of age between 18 to 85 years and both genders were included in this study. Cystoscopy was carried out in all patients using 19F rigid cystoscope under local anesthesia in the department of urology. Barbotage urine sample for LBC was obtained by gently irrigating the bladder with an isotonic saline solution. All patients underwent TURBT of bladder growth and the specimen was sent for histopathology. The Liquid Based Cytology slides were thoroughly examined under the microscope for the various types of cells by a team of consultant pathologists. The observations were recorded and divided into Malignant, Suspicious and Negative for Malignant cells. For practical purposes, suspicious cells were taken as malignant cells. WHO/ISUP classification of urothelial lesions (1998) criteria used for histopathological diagnosis and classification.[10]

RESULTS

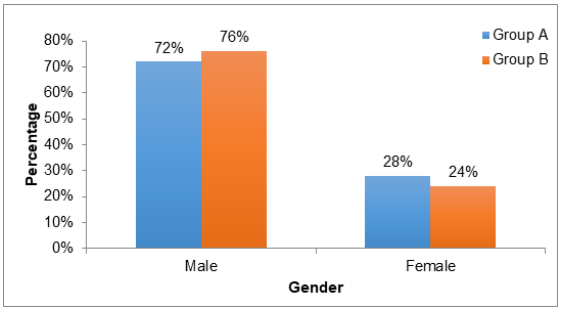
Age and sex distribution in Group A and B

In our study, maximum patients were in the age group of 61-80 years i.e. 11 (44%) in both the groups. The mean age was 61.72±12.92 and 55.04±11.65 years in group A And B respectively, with overall mean age of 58.38±12.63 years. [Graph 1]

In group A there were 18 males (72%) and 7 females (28%). In group B there were 19 males (76%) and 6 females (24%). Out of a total of 50 patients 37 (74%) were males, while 13 (26%) were females. [Graph 2]



Graph 1: Age Distribution



Graph 2: Gender Distribution

Table 1: Distribution of histopathological type of bladder tumor in Group A and B

Diagnosis	GROUP-A (n=25)		GROUP-B (n=25)		Total (%age)
	No. of Patients	%age	No. of Patients	%age	
Non Invasive LG	16	64%	06	24%	22 (44%)
Non Invasive HG	09	36%	19	76%	28 (56%)
Total	25	100%	25	100%	50 (100%)

Table 2: Result of Liquid-based cytology in Group A and B

Result	GROUP-A (n=25)		GROUP-B (n=25)		Total (%age)
	No. of Patients	%age	No. of Patients	%age	
Negative for Malignant Cells	12	48%	7	28%	19 (38%)
Positive for Malignant Cells	13	52%	18	72%	31 (62%)
Total	25	100%	25	100%	50 (100%)

Table 3: Result of Cystoscopy in Group A and B

Result	GROUP-A (n=25)		GROUP-B (n=25)		Total (%age)
	No. of Patients	%age	No. of Patients	%age	
Negative for Malignancy	5	20%	3	12%	8 (16%)
Positive for Malignancy	20	80%	22	88%	42 (84%)
Total	25	100%	25	100%	50 (100%)

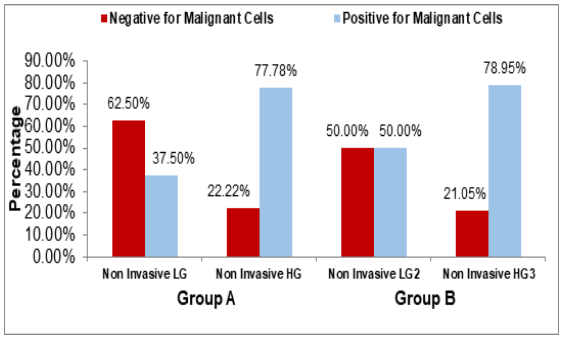
Table 4: Correlation of LBC and Histopathology in Group A and B

Result	GROUP-A		GROUP-B	
	Noninvasive LG	Noninvasive HG	Noninvasive LG	Noninvasive HG
Negative for Malignant Cells	10 (62.5%)	2 (22.22%)	3 (50%)	4 (21.05%)
Positive for Malignant Cells	6 (37.5%)	7 (77.78%)	3 (50%)	15 (78.95%)
Total	16	9	6	19
p value	0.023 (S)		0.010 (S)	

Patients diagnosed with noninvasive low-grade urothelial Carcinoma and noninvasive high-grade urothelial Carcinoma were 64% (16 patients) and 36% (9 patients), respectively in Group A whereas, 24% (6 patients) and 76% (19 patients) in group B respectively. [Table 1]

LBC was positive for malignant cells in 13 patients (52%) in Group A, and 18 patients (72%)in Group B. Out of total 50 patients, LBC was positive for malignant cells in 31 patients (62%). [Table 2]

In group A, 20 (80%) tumors were detected positive for malignancy Whereas, in group B out of 25 patients, 22 (88%) tumors were detected positive for malignancy on final histopathology. A total of 42 (84%) tumors was detected as positive for malignancy. [Table 3]

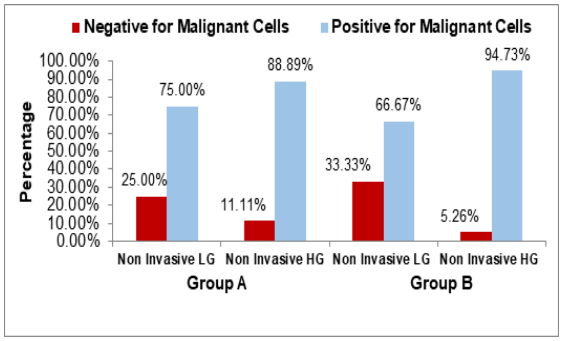


Graph 3: Malignant Cells

In Group A, 6 (37.5%) urine samples showed positivity for malignant cells on LBC out of 16 confirmed patients of noninvasive low-grade urothelial Carcinoma and 7 (77.8%) urine samples out of 9 confirmed patients of noninvasive high-grade urothelial Carcinoma on histopathology. Whereas, In Group B, 3 (50%) urine samples showed positivity for malignant cells on LBC out of 6 confirmed patients of noninvasive low-grade urothelial carcinoma and 15 (78.9%) urine samples showed positivity for malignant cells on LBC out of 19 confirmed patients of noninvasive high-grade urothelial Carcinoma on histopathology. The statistical correlation between LBC and histopathology was significant. (Group A, p valve = 0.023), (Group B, p valve = 0.010). In combined Group A and B, LBC of 9 out of 22 patients showed positivity for malignant cells in patients of noninvasive low-grade urothelial Carcinoma whereas, LBC of 22 out of 28 samples showed positivity for malignant cells in patients of noninvasive high-grade urothelial Carcinoma. The statistical correlation between LBC and histopathology was significant (p valve = 0.007).

Table 4: Correlation of Cystoscopy and Histopathology in Group A and B

Result	GROUP-A (n=25)		GROUP-B (n=25)	
	Noninvasive LG	Noninvasive HG	Noninvasive LG	Noninvasive HG
Negative for Malignancy	4 (25%)	1 (11.11%)	2 (33.33%)	1 (5.26%)
Positive for Malignancy	12 (75%)	8 (88.89%)	4 (66.67%)	18 (94.73%)
Total	16	9	6	19
p value	0.006 (S)		0.037 (S)	



Graph 4: Malignant cells

In Group A, 12 (75%) out of 16 patients showed features of a malignant tumors on cystoscopy in patients of noninvasive low-grade urothelial Carcinoma and 8 (88.8%) out of 9 patients in noninvasive high-grade urothelial Carcinoma. In Group B, 4 (66.6%) out of 6 patients showed features of a malignant tumors on cystoscopy in patients of noninvasive low-grade urothelial Carcinoma and 18 (94.7%) out of 19 patients of noninvasive high-grade urothelial Carcinoma on histopathology. The statistical correlation between cystoscopy and histopathology was significant. (Group A, p valve = 0.006), (Group B, p valve = 0.037).

Overall, 16 out of 22 patients showed features of a malignant tumors on cystoscopy in patients of noninvasive low-grade urothelial carcinoma and 26 out of 28 patients of noninvasive high-grade urothelial carcinoma. The statistical correlation between cystoscopy and histopathology was significant. (p valve =0.024).

The overall sensitivity of LBC was calculated to be 62% with 52% and 72% in Group A and Group B, respectively. The sensitivity of cystoscopy in Group A and Group B was 80% and 88%, respectively with an overall sensitivity of 84%.

Table 5: Statistical analysis of Liquid-based cytology, Cystoscopy and Histopathology

Statistical Parameters	Liquid-Based Cytology			Cystoscopy		
	GROUP-A (n=25)	GROUP-B (n=25)	Overall	GROUP-A (n=25)	GROUP-B (n=25)	Overall
True Positive	13	18	31	20	22	42
False Negative	12	07	19	05	03	08
Sensitivity (%age)	52	72	62	80	88	84
Positive Predictive Value (%age)	100	100	100	100	100	100

DISCUSSION

Age and sex distribution

Age is now widely accepted as the most significant single risk factor for developing bladder carcinoma. Bladder carcinoma can occur at any age but is generally a disease of middle-aged and elderly people. In the present study, total range in 50 patients calculated was 32-86 years and the mean age was 58.38±12.63 which was comparable with the studies done by Gupta et al.[11] (2009), Zhang et al.[12] (2012), Pudasaini et al,[13] (2014), Aliramaji et al,[14] (2015) and Prakash et al,[15] (2019). In various demographic studies, it has been seen that individuals aged ≥65 years have 11 times the incidence of cancer in general and a 15-times higher cancer mortality rate than individuals aged <65 years.[16] With advancing age, specific genes might be activated while others are suppressed. An aged cell might have a decreased capacity for repair of mutations in its DNA.[17-19]

In the present study, 37 (74%) were males and 13 (26%) were females which were comparable with the studies done by Zhang et al,[12] (2012), Pudasaini et al,[13] (2014), Aliramaji et al,[14] (2015) and Prakash et al,[15] (2019). Higher incidence and severity of bladder carcinoma in males are attributed to excessive environmental exposure to carcinogens, such as tobacco and industrial chemicals in men as compared to women.[20,21]

Histopathological findings

In the present study, 28 (56%) patients were diagnosed as high-grade nonmuscle invasive carcinoma and 22 (44%) patients were diagnosed as Low-grade nonmuscle invasive carcinoma. This result was comparable to the study conducted by Sharma et al,[22] (2016), which showed high-grade carcinoma as 74% and low grade as 44%. Sathya et al,[23] in 2014, which showed 44/70 (63%) of high grade and 17/70 (24%) of low grade carcinoma. Another study conducted by Pudasaini et al,[13] (2014) showed noninvasive papillary urothelial Carcinoma, low grade (29%) and noninvasive papillary urothelial carcinoma, high degree (6.5%).

Findings of Liquid-based cytology and its correlation with histopathology. In Group A LBC samples of 13 patients (52%) were positive for malignant cells, whereas in group B LBC samples of 18 patients (72%) were positive. Out of total 50 patients, LBC samples of 31 patients (62%) were found to be positive for malignant cells. 22 out of 28 (78.5%) high-grade urothelial carcinoma patients showed cancerous cells on LBC and 9 out of 22 (41%) low-grade urothelial carcinoma patients were positive for cancerous cells on LBC. The overall sensitivity of LBC was calculated to be 62% with 52% and 72% in Group A and Group B, respectively.

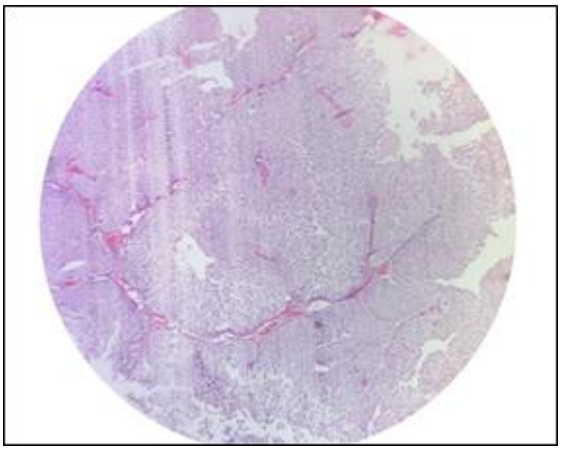


Figure 1: H and E Stained Slide shows papillary fronds lined by Urothelium displaying minimum atypia in low-grade nonmuscle Invasive Urothelial Carcinoma (x100)

Based on previous studies, overall sensitivity of LBC ranges from 21% to 80%.[9] In 2015 results of meta anylasis by Luo et al,[9] showed that the pooled sensitivity of LBC was 58% (51%-65%). In 2018, XU et al,[24] concluded that the sensitivity of LBC was 77.8% in the detection of bladder carcinoma. The lowest sensitivity of LBC was detected by Sullivan et al,[25] in 2009. They found that overall sensitivity of liquid-based cytology for urothelial cell carcinoma was 21%. In 2014, Piaton et al,[26] found out 80% sensitivity. In a study conducted by Son et al,[9] (2012) showed positive cytology in 29.4% of low-grade urothelial carcinoma confirmed on histopathology while urinary cytology was positive in 60.6% of high-grade urothelial carcinoma

confirmed on histopathology. The overall sensitivity of LBC being 50%. A study conducted by Chawla et al,[5] (2018) showed 38.1% of cases as malignant by LBC, out of a total 110 cases of urothelial Carcinoma. The sensitivity of cytology in the detection of recurrence was 61% by Tahoun et al,[27] (2010).

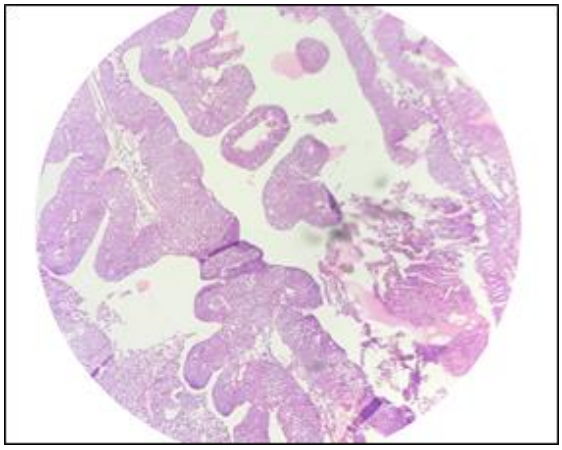


Figure 2: H and E Stained Slide shows high-grade nonmuscle-invasive urothelial carcinoma (x100)

The main factors for improved sensitivity of LBC over conventional cytology is due to its several advantages such as Uniformly distributed and cell-enriched slides, Less obscuring elements in the background, Thin-layered cells require less time to make a diagnosis, reduction in the slide-making time, obliteration of nonurothelial cells and mucus in the urine, humidified slides, also decreased cell degeneration by a preservation solution, and improved slide quality.[28] However, LBC may reduce the probability of atypical urothelial cells on the slide, which can be the cause unstable sensitivity.[29]

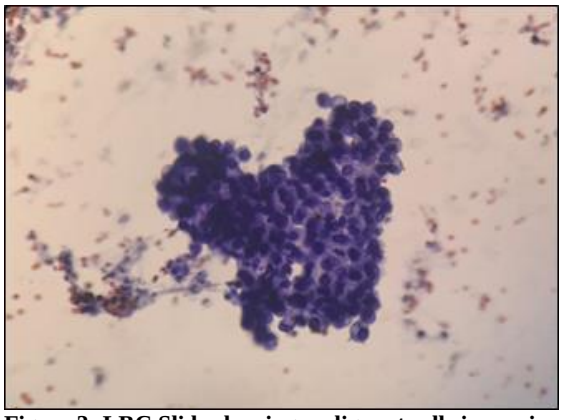


Figure 3: LBC Slide showing malignant cells in a urine sample

Findings of cystoscopy and its correlation with histopathology

Cystoscopy allows direct visualization of the bladder mucosa and has the pivotal role for detecting primary and recurrent urothelial Carcinoma but it remains an invasive procedure. Exophytic tumors are reliably diagnosed as compared to flat transitional cell carcinoma, particularly Carcinoma in situ. Therefore, exfoliative urinary cytology and barbotage specimens are routinely performed to complement cystoscopy.[30]

In our study 20 (80%) out of 25 patients and 22 (88%) out of 25 tumors showed features of malignancy in groups A and B, respectively. Out of 50 cases, 42 (84%) tumors were detected as positive for malignancy. The sensitivity of cystoscopy in Group A patients is 80% and in Group B is 88%.while, the overall sensitivity of total 50 patients was 84%. A study conducted by Gupta et al,[1] (2019) showed 39 (100%) patients as malignant out of total 39 patients of urothelial Carcinoma confirmed histologically. Another study conducted by Soubra et al,[31] in 2015 showed sensitivity of cystoscopy as 85–90%. Gupta et al,[11] (2009) also concluded that 6/60 cases (76.6%) were neoplastic. The sensitivity of cystoscopy in the detection of recurrence was 78% by Tahoun et al,[27] (2010).

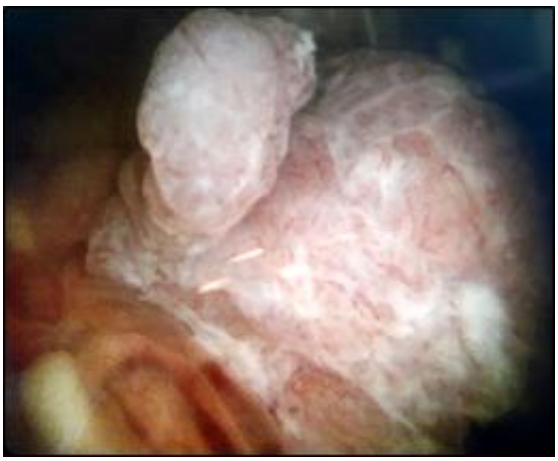


Figure 4: Cystoscopy of Bladder tumor showing Solid growth

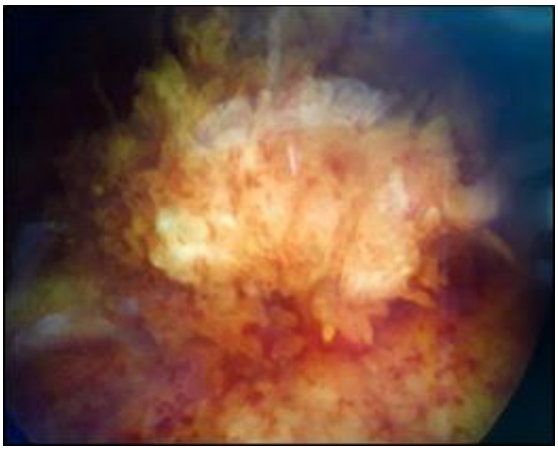


Figure 5: Cystoscopy of Bladder tumor showing papillary growth

Comparison of Liquid-based cytology and cystoscopy with histopathology

Cystoscopy is invasive, but still, its diagnostic precision is higher than that of noninvasive methods such as urine cytology. In contrast, it is inferior to cytological examination in recognizing flat transitional cell carcinoma and in the assessment of the extent of disease remaining or recurrent after treatment.[32]

In the present study, the cystoscopy diagnosed more number of positive cases (84%) as compared to Liquid-based cytology (62%), which was comparable to the survey conducted by Tahoun et al,[27] (2010). It was observed that the sensitivity of LBC increased significantly with the grade of malignancy. This is explained that due to the cohesive nature of low-grade tumors, they are not routinely shed into the urine and these cells have similar cytomorphology to healthy urothelial cells.[25]

Studies (Authors and Year)	Positive on LBC	Positive on cystoscopy	Positive on Histopathology
Tahoun et al,[44] (2010)	25	32	41
Present Study	31	42	50

CONCLUSION

Cystoscopic examination alone has limited sensitivity for tumors that are not visualized, such as Carcinoma in situ. Barbotage specimens and exfoliative urinary cytology should be performed to complement cystoscopy. LBC has better sensitivity than conventional urine cytology as there occurs better cell preservation. It is a simple, noninvasive screening test for screening and detection of early cases of bladder cancer. The sensitivity of urinary cytology increases with tumor grade. So, It is useful in monitoring of patients with high grades, superficial urothelial Carcinoma and Carcinoma in situ. Despite high sensitivity of urinary cytology and cystoscopic examination of bladder mass, histopathological diagnosis remains essential because it allows definitive diagnosis, a grade of the tumor, assessment of the degree of differentiation, Tumour staging.

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