ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Efficacy of categorization of urothelial malignancy in atypical urine samples by the Paris system for reporting urinary cytology

Thameem A[1], Sankar Sundaram[2], Deepa Sujatha[3]

[1]Junior Resident, [2]Professor, [3]Associate Professor CAP, Department of Pathology, Government Medical College, Kottayam, Kerala, India.

Submission: 11-10-2022 Revision: 02-04-2023 Publication: 01-05-2023

ABSTRACT

Background: Urine cytology is an important screening tool of patients for urothelial carcinoma and follow-up of patients. Ease of procurement, cost-effectiveness, and lower turnaround time are the advantages. **Aims and Objectives:** The aim of the study was to identify the accuracy of TPS criteria in diagnosing HGUC, considering biopsy as the gold standard and to describe the cytomorphological features in atypical urine specimens and categorization as per reference to TPS of reporting urine cytology. Definition – Atypical urine sample: Urine samples of patients with macroscopic hematuria and other unexplained urinary symptoms, suspicious of urothelial malignancy. **Materials and Methods:** The study was performed on patients attending urology OPD with macroscopic hematuria and other unexplained urinary symptoms, which were suspicious of urothelial malignancy and whose urine cytology and subsequent biopsy specimens were submitted to the Department of Pathology. Continuous sampling of 56 patients was done. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were used to compare TPS with histopathology. SPSS was used to assess correlation, done by diagnostic test evaluation. **Results:** Age group of study population ranges from 40 to 84 years with mean age of population as 62 years. Sensitivity, specificity, PPV, and NPV of TPS for reporting urinary cytology in the investigation were 85%, 75%, 65.38%, and 90%. Combination of elevated nuclear-to-cytoplasmic ratio, irregular nuclear border, and hyperchromasia was predictive of malignancy. **Conclusion:** The criteria outlined by TPS facilitated the standardization of urine cytology reporting and significantly increased the sensitivity of diagnosing HGUC.

Key words: The Paris system; Urine cytology; Bladder biopsy; High grade urothelial carcinoma

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v14i5.48836

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

Cytology of urine sample introduced by Dr. Papanicolaou in 1945 is still used as a non-invasive, easily obtained, and cheap diagnostic modality in the screening of urothelial cancers. Urine specimen for cytology study reaches clinical pathology laboratory in large numbers and is useful to the urologists in the detection of urothelial malignancy as a supplement to radiology and endoscopy. Till date, diagnosing atypia on urine cytology remains a significant challenge in urine cytology. Due to lack of uniformity in the terminology and diagnostic criteria, the reporting of a typical cells in urine specimens remains widely variable. Two strategies have been introduced recently to improve urine cytology reporting, which include: (1) Reduction in the number of indeterminate diagnoses, and (2) to define each category, especially atypia in a standardized manner. "The Paris system (TPS) for reporting urine cytology" is the result of the same.[1] This reporting system is intended mainly to detect high-grade urothelial carcinoma (HGUC) on cytology and minimize the detection of low-grade urothelial carcinoma (LGUC), the sensitivity being high for the former and questionable for the latter. The LGUC lesions yield very few cells and these cells closely resemble

Address for Correspondence:
Dr. Deepa S, Associate Professor CAP, Department of Pathology, Government Medical College, Kottayam, Kerala, India.
Mobile: +91-9447526445. **E-mail:** drdeeps@gmail.com

normal benign urothelium in morphology. Hence, a universal morphological criterion could not be made, explaining the low sensitivity in diagnosing LGUC.

This study is done based on TPS for reporting urinary cytology to assess the performance and utilization of morphological features in the urine cytology smears in diagnosing HGUC. The objectives of this study are to identify the accuracy of TPS criteria in diagnosing HGUC, considering biopsy as the gold standard and to describe the cytological findings in atypical urine specimens and categorization as per reference to TPS.

Aims and objectives

The aim of the study was to identify the accuracy of TPS criteria in diagnosing HGUC, considering biopsy as the gold standard and to describe the cytomorphological features in atypical urine specimens and categorization as per reference to TPS of reporting urine cytology.

MATERIALS AND METHODS

Ours were an interventional diagnostic test evaluation study conducted at Government Medical College's cytology division under the Department of Pathology. A total of 56 urine specimen was studied after sample size calculation based on the study by Glass et al., 2017.[3]

Inclusion and exclusion criteria

Adequacy of the sample, that is, presence of atypical cell/benign urothelial cells/>25 mL sample volume and patients who had/had not undergone surgery or other treatment for the neoplasm on surveillance were the inclusion criteria in the study. Patients with urinary symptoms due to definite non-neoplastic etiology were excluded from our study.

Patients with macroscopic hematuria and other unexplained urinary symptoms were identified from. The urology ward and their urine cytology analysis was carried out with a subsequent study of their bladder biopsy. Detailed clinical information including age, sex, and imaging details was taken. Urine samples and/or bladder wash were collected on 3 consecutive days and submitted to the department of cytology in all suspicious cases of urothelial carcinoma. Measures were taken to process urine afresh. Cytology numbers were documented and the slides were stored. Urine samples were discarded after the preparation of smears. Smears were prepared after cytocentrifugation of the urine sample.

Cytocentrifugation is done in 1500 rpm for 10 min. A thin layer of smear is formed on the glass slide.[5] The smears are immediately fixed in 95% ethanol for pap staining whereas air-dried smears are Giemsa stained. Both Pap and Giemsa-stained smears were studied in each case and reporting was done based on TPS. A pathologist who had no access to additional biopsy or cytology results reported the slide. The individual cytomorphological features were described.

The cases were followed up with their biopsy. A biopsy requires trained medical professionals, and sophisticated and elaborate routine histopathological processing before making its way into slides handled by a pathologist, paving the way to diagnosis.

The accuracy of diagnosing gigh-grade urothelial carcinoma in urine/bladder wash cytology was compared with histopathology, being the gold standard. Subsequently, the cytomorphological feature in isolation and in combination, contributing to the diagnosis was analyzed.

Frequencies of HGUC detected by cytology as per reference to TPS for reporting urinary cytology and histopathology were examined.

Variables taken were nuclear chromatin, nuclear borders, and nucleocytoplasmic ratio (N/C ratio). Correlation of HGUC diagnosed by cytology as per reference to TPS for reporting urinary cytology as compared to histopathology was exercised. Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV) of TPS for reporting urinary cytology and sensitivity and specificity of cytomorphological features in detecting high-grade urothelial carcinoma was compared with histopathology being the gold standard.

RESULTS

Of the total 56 patients included in the study, 55.35% of patients submitted voided urine for cytology study and remaining 44.65% were bladder wash. Of the 56 cases studied, 50 were males comprising 89.28 % of the cases. The mean age of the present study population was 62 years. Majority belonged to age groups of 61–70 years with 21 patients, that is, 37.5%. Most common clinical presentation was painless gross hematuria in 32 cases followed by dysuria in 24 cases. Of the 56 cases, 18 cases (32%) were given a cytological diagnosis negative for HGUC (TPS II category). Fourteen cases (25%) were offered a cytology diagnosis suspicious for HGUC (SHGUC) (TPS IV). Twelve cases (21%) were given a cytology diagnosis of HGUC (TPS V) and remaining seven cases were consistent with TPS Category III showing atypical urothelial cells (Table 1). Cytohistopathological correlation was made in 56 cases. One case diagnosed as unsatisfactory (TPS I), turned out to be high-grade papillary urothelial carcinoma in histopathology. Of the 18 cases with a

cytology diagnosis of TPS II, histopathology follow-up was HGUC and non-invasive low-grade urothelial neoplasm (Non-invasive LGUN) in two cases each followed by one case each of transitional cell carcinoma ureter (TCC) and LGUN (Table 2). Remaining cases were benign which included a case each of cystitis and tuberculosis (Table 3 and Figure 1). Among seven cases of TPS III, four cases were negative for malignancy in histopathology. Of the remaining four cases, TCC ureter,[1] non--invasive LGUN,[2] and HGUC[1] were the histopathology. Of the 14 cases with cytology diagnosis of TPS IV – SHGUC, HGUC was the histopathology diagnosis in six cases followed by TCC, non-invasive LGUN, and LGUC in three cases each. Only one case turned out to be benign (cystitis cystica). Of the 12 cases with cytology diagnosis of TPS V, 11 turned out (Figure 2) to be HGUC on biopsy, the 12th case being diagnosed as non-invasive LGUC (Figure 3).

DISCUSSION

Neoplasm of urinary bladder is very common and is the second most common malignancy in males following prostatic malignancy. Tobacco, occupational exposure to chlorinated hydrocarbons, and ionizing radiation exposure are some of the known risk factors. Diagnosis of urothelial tumors can be made combining detailed clinical findings with radiology, urine cytology, and histopathology. Urine cytology is a very common and easy screening test widely (Figure 1) performed in the diagnosis of urothelial malignancy with a high sensitivity for high-grade urothelial neoplasms.

The bladder urothelium is the most studied urothelium. Although the surface of urinary bladder undergoes changes during varying phases of micturition, the bladder urothelium performs important biologic functions. It forms a stable apical surface, providing effective permeability barrier. The factors that allow the bladder urothelium to perform these tasks are the presence of effective tight junctions, increased uroplakin, and slow urothelial cell turnover which takes around 200 days[10] The superficial urothelium is constituted by a monolayer of large multinucleated and highly differentiated superficial umbrella cells which contain abundant uroplakin that form urothelial plaques. Smaller and less well-differentiated epithelial cells form the intermediate and basal cell layers of the urothelium. Urothelial stem cells are seen in the basal cell layer[2,3] The intermediate cells and basal cells serve as a reservoir for rapid umbrella-cell regeneration. Urine cytology possesses advantages, it being non-invasive and devoid of instrumentation artifact. It is cost-effective and not time demanding devoid of learning curve.[13] Bladder wash was collected from patients randomly, with an urge for an increased cellular yield. Cytospin preparation of urine was used that provided greater cell yield and showed monolayer arrangement of cells in the slide for microscopy. The technique poses a learning curve.[14] Primary or recurrent urothelial carcinoma can be diagnosed by the presence of exfoliated cells in the urine or bladder washing having malignant cytological features.[9] Studies show no relevant difference between voided urine and bladder washing samples. Hence, it is recommended to use the sample that is most readily available locally if cytology is indicated.[8] Voided urine-based quantitative cytology can be implemented in daily practice if sample is correctly processed.[9] Absence or lack of adequate number of urothelial cells identified on the smeared slide is the reason why majority of inadequate and suboptimal specimens are classified as such; Inadequate or small volume of urine

Table 1: Cytological diagnosis n=56

Category	Diagnosis	Number of cases	Percentage
TPS I	Unsatisfactory	5	9
TPS II	Negative for HGUC	18	32
TPS III	Atypical urothelial cells	7	13
TPS IV	SHGUC	14	25
TPS V	HGUC	12	21
TPS VI	LGUN	-	0
TPS VII	Other malignancies	-	0
Total		56	100

HGUC: High-grade urothelial carcinoma, SHGUC: Suspicious for HGUC, LGUN: Low-grade urothelial neoplasm, TPS: The Paris system

Table 2: Comparison of cytological diagnoses with histopathological diagnoses n=56

	Histopathology diagnosis								
TPS	Cystitis	Cystitis cystica	Tb	Negative for malignancy	TCC ureter	Non-invasive LGUC	Invasive LGUC	HGUC	Total
I								1	1
II	3		1	9	1	2	1	2	19
III				4	1	2		1	8
IV		1			3	3	3	6	16
V						1		11	12
VI									
VII									
Total	3	1	1	13	5	8	4	21	56

HGUC: High-grade urothelial carcinoma, LGUC: Low-grade urothelial carcinoma, TCC: Transitional cell carcinoma

Table 3: Histopathological diagnosis n=56

S. No.	Diagnosis	Total	Percentage
1.	Cystitis	3	5
2	Cystitis cystica	1	2
3	Tuberculosis	1	2
4	Negative for malignancy	13	23
5	Non-invasive LGUC	8	14
6	Invasive carcinoma LGUC	4	7
7	HGUC	21	38
8	TCC ureter	5	9
	Total	56	100

HGUC: High-grade urothelial carcinoma, LGUC: Low-grade urothelial carcinoma, TCC: Transitional cell carcinoma

submitted for study is the main reason for reports such as inadequate and suboptimal specimens. The prevalence of malignant and suspicious findings is lower in low-volume specimens than in high volume specimens. Although exact cutoff levels are problematic, 30 mL of urine or bladder washing may be considered a reasonable required volume to minimize the number of inadequate and suboptimal specimens. Specimen volume <10 mL significantly reduce the chance of diagnosing high-grade urothelial carcinoma. Studies show that patients with a history of inadequate or suboptimal voided urine specimen usually yield an adequate specimen if they return for a repeat specimen within 6 months of the original specimen and provide an adequate volume of urine sample (>30 mL).[11] Quantitative cell recovery is not routinely needed and very acceptable results may be obtained with routine procedures.[7]

In the present study, cytological examination of urine samples of 56 patients having symptoms suggestive of bladder tumors was studied and diagnosis made based on the criteria proposed by TPS for reporting urinary cytology. This study was aimed at evaluating the accuracy of TPS criteria in diagnosing HGUC in atypical urine samples, considering biopsy as the gold standard. In our study, majority (n-50) patients were of male gender. which is in accordance with a study conducted by Boring CC et al., (1994).[13] Majority of the patients (n-21) belonged to age groups of 61–70 years, that is, 37.5%. Bladder cancers are more common in 6th and 7th decade.[15] People inflicted with HGUC belong to age group 51–80, with 45% cases being in age group 71–80 years. In the study, incidence of HGUC below 50 years and above 80 years was nil. Median age at diagnosis of HGUC in our study was 71–80 years. In the present study, there were 20 cases of HGUC. All the affected were males with no females inflicted, in our study. The male preponderance is seen in a study conducted by Boring CC et al., (1994).[13]

Among 21 HGUC cases in the present study, 19 had presented with hematuria and only two patients presented with lower urinary tract symptom (dysuria). Hence, most common symptom of bladder cancer is hematuria.[16]

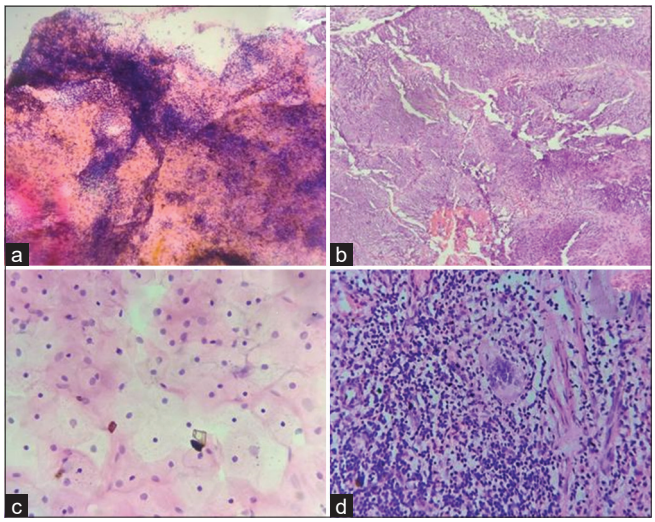

Figure 1: (a) cytology – TPS I Pap Stain ×10, (b) Histopathology – HGUC H and E ×40, (c) cytology – TPS II Pap Stain ×40, (d) histopathology – Follicular Cystitis H and E ×40

Cytohistopathological correlation was made in 56 cases. One case diagnosed as unsatisfactory (TPS I), turned out to be high-grade papillary urothelial carcinoma in histopathology. TPS describes a risk of malignancy (ROM) of 5–10%. Among the 18 cases diagnosed as NHGUC, there were cases of LGUC, HGUC and TCC in histopathology. TPS describes a ROM <10% in TPS II.

Among seven cases of TPS III, four cases were negative for malignancy in histopathology. These cases on retrospective understanding were found to have lithiasis. The reactive atypia was over diagnosed as TPS III. In urinary lithiasis, the nuclei of urothelial cells can be enlarged and pleomorphic, irregular in size and shape, with increased N: C ratios. The chromatin can be coarse and hyperchromatic with prominent nucleoli. Mitotic figures can be seen. Necrosis, inflammation, and blood may be seen in the background, presenting a similitude to a tumor diathesis. Hence, reactive changes can closely mimic malignancy.[12] One false positive case in cytology, which was diagnosed as suspicious of high-grade urothelial carcinoma, but found to be cystitis cystica in histopathology could be due to degenerative atypia that mimicked HGUC. Bladder calculi, urinary obstruction, diabetes mellitus, instrumentation, and immune deficiency are a few of the risk factors for cystitis. Irradiation of the bladder region may causes radiation cystitis.[3,4]

On describing the cytomorphological features in atypical urine samples by TPS, high N: C ratio, hyperchromasia and irregular nuclear border were found to have a high sensitivity in diagnosing HGUC, in the present study (Table 4). It is the objective cytomorphological description, but not the subjective cytology analysis that help to reach the right diagnosis. Similarly, the study by Glass et al., found that identification of high N: C ratio along with other features

Table 4: Sensitivity and specificity of cytomorphological features in diagnosing HGUC

Feature	Sensitivity		Specificity	
	Present study	Glass et al.,[3] n=118	Present study	Glass et al.,[3]
High n: c ratio	85	68	52	48
Irregular nuclear border	85	90	75	40
Hyperchromasia	85	94	52	46
Coarse granular chromatin	45	64	88	57
High n: c+irregular nuclear border	8	64	77	67
High n: c+coarse granular chromatin	45	53	88	72

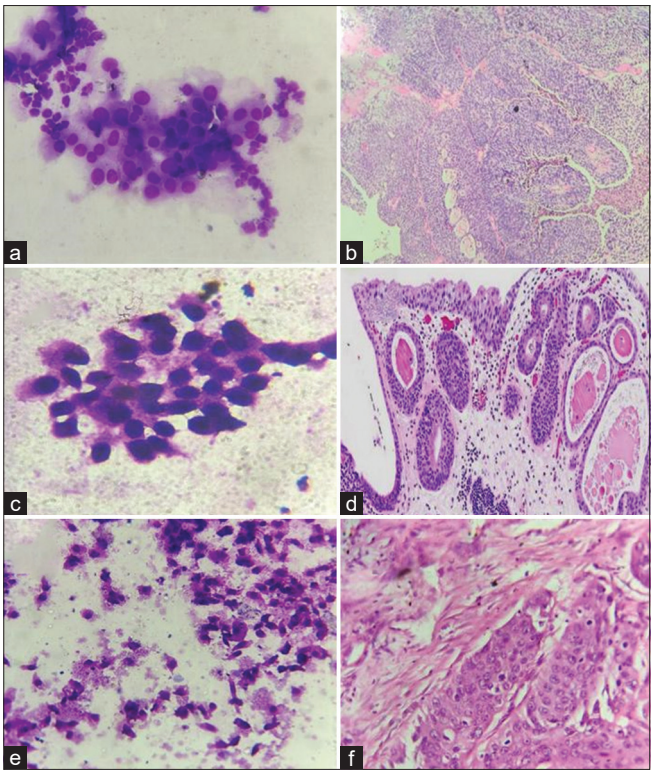

Figure 2: (a) cytology – TPS III Giemsa ×100 (b) histopathology – LGUN H and E ×40 (c) cytology – TPS IV Pap Stain ×40 (d) histopathology – Cystitis Cystica H and E ×40 (e) cytology TPS V PAP Stain ×100 (f) Histopathology HGUC H and E ×40

was more predictive of malignancy.[19] Piaton et al., described nuclear hyperchromasia and increased nucleocytoplasmic ratio as the most informative diagnostic criteria.[20] Bhatia et al., concluded that nuclear features such as high N: C ratio, hyperchromasia, and chromatin abnormalities were useful for assessing the malignant cells[13] Sensitivity and specificity of TPS in detection of HGUC in our study are 85% and 75%, Respectively, which is in accordance with the study conducted by Rai et al., in 2019 which shows 83% sensitivity and 84% specificity.[16] PPV and NPV in our study are calculated as 65.38% and 90%, respectively. In the present study, all urothelial malignancies could not be diagnosed by urine cytology alone. False negativity may also be due to inadequacy of screening or since some of urothelial carcinoma, such as low-grade tumors rarely shed malignant cells.

Limitations of the study

The sample size was small. There are a few limitations found in our study. All cases of hematuria or other unexplained urinary symptoms, who submitted urine/bladder wash to the department of cytology did not undergo tissue diagnosis. They were kept on routine follow-up. Some patients were lost to follow-up, as they approached other care centers in their follow-up visits.

CONCLUSION

Urine cytology in atypical urine samples shows fairly high diagnostic accuracy for the detection of urothelial carcinoma with a high sensitivity for HGUC.[18] Accuracy in diagnosing malignancy by an experienced pathologist is highly dependent on the diagnostic cell yield in the sample along with technique of sample processing. The TPS criteria for reporting urine cytology is aimed to standardize the reporting of urinary tract cytology, defining specific parameters for diagnosing atypical urothelial cells and HGUC. Analysis shows that three features, that is, high N: C ratio, hyperchromasia and irregular nuclear border are having higher sensitivity in identifying HGUC.

ACKNOWLEDGMENT

We thank Kerala University of Health Sciences and Government Medical College Kottayam, Kerala, India, for having given us the opportunity to perform and complete the study.

REFERENCES

1. Ghosh A. The Paris system-a new insight into reporting urine cytology. J Pathol Nepal. 2016;6(11):953-958. https://doi.org/10.3126/jpn.v6i11.15681
2. Ho PL, Kurtova A and Chan KS. Normal and neoplastic urothelial stem cells: Getting to the root of the problem. Nat Rev Urol. 2012;9(10):583-594. https://doi.org/10.1038/nrurol.2012.142
3. Glass R, Rosen L, Chau K, Sheikh-Fayyaz S, Farmer P, Constantinos C, et al. Analysis of the cytomorphological features in atypical urine specimens following application of the Paris system for reporting urinary cytology. Acta Cytol. 2018;62(1):54-61.

https://doi.org/10.1159/000481278

4. Parkin CJ, Acland G, Sulaiman B, Johnsun ML and Latif E. Malakoplakia, a malignant mimic. Bladder (San Franc). 2020;7(3):e44.
   https://doi.org/10.14440/bladder.2020.818
5. Linder J. Recent advances in thin-layer cytology. Diagn Cytopathol. 1998;18(1):24-32.
   https://doi.org/10.1002/(sici)1097-0339(199801)18:1<24:aid-dc5>3.0.co;2-u
6. Keller AK and Jensen JB. Voided urine versus bladder washing cytology for detection ofurothelial carcinoma: Which is better? Scand J Urol. 2017;51(4):290-292.
   https://doi.org10.1080/21681805.2017.1310130
7. Moonen PM, Peelen P, Kiemeney LA, Boon ME, Schalken JA and Witjes JA. Quantitative cytology on bladder wash versus voided urine: A comparison of results. Eur Urol. 2006;49(6):1044-1049, discussion 1049-1050.
   https://doi.org/10.1016/j.eururo.2006.01.029
8. Ahmed HG and Tom MA. The consequence of delayed fixation on subsequent preservation of urine cells. Oman Med J. 2011;26(1):14-18.
   https://doi.org/10.5001/omj.2011.04
9. VandenBussche CJ, Rosenthal DL and Olson MT. Adequacy in voided urine cytology specimens: The role of volume and a repeat void upon predictive values for high-grade urothelial carcinoma. Cancer Cytopathol. 2016;124(3):174-180.
   https://doi.org/10.1002/cncy.21634
10. Beyer-Boon ME, Cuypers LH, de Voogt HJ and Brussee JA. Cytological changes due to urinary calculi: A Consideration of the relationship between calculi and the development of urothelial carcinoma. Br J Urol. 1978;50(2):81-89.
    https://doi.org/10.1111/j.1464-410x.1978.tb03031.x
11. Highman W and Wilsonv E. Urine cytology in patients with calculi. J Clin Pathol. 1982;35(3):350-356.
    https://doi.org/10.1136/jcp.35.3.350
12. Kim YJ and Kim HJ. A Comparison between ThinPrep monolayer and cytology for the detection of bladder cancer. Korean J Urol. 2014;55(6):390-394.
    https://doi.org/10.4111/kju.2014.55.6.390
13. Boring CC, Squires TS and Tong T. Cancer statistics, 1991. CA Cancer J Clin. 1991;41(1):19-36.
    https://doi.org/10.3322/canjclin.41.1.19
14. DeGeorge KC, Holt HR and Hodges SC. Bladder cancer: Diagnosis and treatment. Am Fam Physician. 2017;96(8):507-514.
15. Amin MB, McKenney JK, Paner GP, Hansel DE, Grignon DJ, Montironi R, et al. ICUD-EAU international consultation on bladder cancer: Pathology. Eur Urol. 2012;63(1):16-35.
    https://doi.org/10.1016/j.eururo.2012.09.063
16. Rai S, Lali BS, Venkataramana CG, Philipose CS, Rao R and Prabhu GL. A quest for accuracy: Evaluation of the Paris system in diagnosis of urothelial carcinomas. J Cytol. 2019;36(3):169-173.
    https://doi.org/10.4103/JOC.JOC_67_18
17. Rohilla M, Singh P, Rajwanshi A, Gupta N, Srinivasan R, Dey P, et al. Cytohistological correlation of urine cytology in a tertiary center with application of the Paris system. Cytopathology. 2018;29(5):436-443.
    https://doi.org/10.1111/cyt.12604
18. Pastorello RG, Barkan GA and Saieg M. Experience on the use of the Paris system for reporting urinary cytopathology: Review of the published literature. J Am Soc Cytopathol. 2021;10(1):79-87.
    https://doi.org/10.1016/j.jasc.2020.10.002
19. Piaton E, Decaussin-Petrucci M, Mege-Lechevallier F, Advenier AS, Devonec M and Ruffion A. Diagnostic terminology for urinary cytology reports including the newsubcategories 'atypical urothelial cells of undetermined significance' (AUC-US) and 'cannot exclude high grade' (AUC-H). Cytopathol. 2014;25(1):27-38.
    https://doi.org/10.1111/cyt.12050
20. Bhatia A, Dey P, Kakkar N, Srinivasan R and Nijhawan R. Malignant atypical cell in urine cytology: A diagnostic dilemma. Cytojournal. 2006;3:28.
    https://doi.org/10.1186/1742-6413-3-28

Authors' Contributions:
TA- Concept and design of the study, prepared first draft of manuscript, statistical analysis; **DS-** Interpreted the results; reviewed the literature and manuscript preparation; **SS-** Concept, coordination, and interpretation, preparation of manuscript and revision of the manuscript.

Work attributed to:
Department of Pathology, Government Medical College, Kottayam - 686 008, Kerala India.

Orcid ID:
Dr. Thameem A - https://orcid.org/0000-0002-3820-1719
Dr. Sankar Sundaram - https://orcid.org/0000-0002-5707-6423
Dr. Deepa Sujatha - https://orcid.org/0000-0002-7805-6624

Source of Funding: Nil, **Conflicts of Interest:** None declared.