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Evaluation of Urine Cytology in Urothelial Carcinoma with respect to the Paris System for Reporting Urinary Cytology

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Abstract
Objectives: *This study aims to evaluate urine cytology as an investigational tool for urothelial carcinomas of urinary bladder in the light of the Paris System for reporting Urinary Cytology, the main goal being utilizing the diagnostic accuracy of this system in detection of high grade urothelial carcinoma.*
Methods: *A cross-sectional observational study was conducted over 1 year (August 2016- July 2017). The study population comprised patients presenting with haematuria and scheduled for trans-urethral resection of bladder tumour or radical cystectomy after a preliminary cystoscopy. Preoperative, early morning, midstream, voided urine samples were collected and processed. Reporting of urine cytology was done according to the Paris System. Results were correlated with gold standard test i.e. Histopathology. Test thresholds were selected for cytology and histopathology. Relevant statistical tests were utilized for data analysis.*
Results: *72 cases were studied. According to the reporting criteria of the Paris System, 66.66% cases were positive for malignancy. 83.33 % of the positive and 75% of the negative cases were confirmed as such by histopathology. As per concordance between cytology and histopathology, the p value calculated was 0.0000571. The sensitivity of urine cytology was calculated to be 86.95%, the positive predictive value was 83.33%. The likelihood ratio of urine cytology as reported by the Paris system in diagnosing positive result in high grade carcinoma was calculated to be 2.82.*
Conclusion: *Urine Cytology as reported by the Paris System will lead to uniform and unequivocal reporting of urothelial carcinoma.*
Keywords: *Urine Cytology; Paris System; Urothelial Carcinoma.*

Introduction

Urothelial carcinoma is the fourth most common non dermatological malignancy in men, with a male: female preponderance of 3:1.[(1)] The pattern of growth may be exophytic or endophytic, or a combination of both. Stromal invasion by

urothelial carcinoma proceeds in two stages: invasion of the lamina propria and invasion of the muscle layer.

Urologists over decades have accepted the limitations of cystoscopy as a diagnostic method, more so for flat urothelial lesions.[2] In spite of urine cytology having the advantage of being an inexpensive, simple and non-invasive investigational tool, it has been under scrutiny due to a wide range of sensitivity depending on the grade of the tumour and the questionable standards of specimen collection, preservation, processing as well as a uniform reporting system in the past. However, cytologic analysis has high accuracy for both superficial high grade lesions as well as those extending deep into the bladder wall.[3]Hence, for practical use, a flexible, concise yet specific system of terminology was needed to report urine cytology.

The idea of developing The Paris System for Reporting Urinary Cytopathology was conceived during the International Academy of Cytology Congress held in Paris in May 2013. During the 2013 International Congress of Cytology in Paris, interested stakeholders like the International Academy of Cytology and the American Society of Cytopathology concluded that a standardized system for reporting urinary cytology should be established. In late 2015, the consensus group published their guidelines, known as The Paris System (TPS) for Reporting Urinary Cytology.[4]

The diagnostic categories for reporting urine cytology as stated in The Paris System included the following:

- Non diagnostic or unsatisfactory
- Negative for High Grade Urothelial carcinoma
- Atypical Urothelial Cells
- Suspicious for High Grade Urothelial Carcinoma
- High Grade Urothelial Carcinoma
- Low Grade Urothelial Neoplasms

The preservation of urothelial cells in voided urine and hence the adequacy has always been a reason of major drawback of urine cytology, more so due to the lack of a satisfactory system, stating guidelines of adequacy. The Paris System (TPS) stated the criteria of at least 20 well preserved and well visualized urothelial cells per 10 high power fields, in the absence of obscuring lubricant, inflammatory cells or red blood cells, for stating the sample as *adequate*. This paper aims to evaluate the accuracy of urine cytology as reported according to the terminologies, categories and diagnostic criteria stated in The Paris System, correlation being with the histopathology of the cases selected.

Materials & Methods

The study was carried out for a period of 1 year starting from August 2016 to July 2017. The study population included all patients attending the Department of Urosurgery with symptoms of haematuria or dysuria, who were planned for some surgical procedure- either Trans Urethral Resection of Bladder Tumours (TURBT) or radical cystectomy after a preliminary cystoscopic examination. A total of 72 cases were collected during the study period after they were admitted to the Department of Urosurgery.

In each case, in the pre operative period, three subsequent early morning, midstream, voided urine samples were collected on three subsequent days. In order to avoid degeneration of cells, centrifugation of the sample was done immediately at 2000 rpm for 10 minutes. Smears were made from the sediment on clean, grease free glass slides and were fixed in 95% alcohol for about 3-4 hours. The smeared slides were stained with Papanicolaou stain and reported as per The Paris System. The sample was called inadequate only when it did not show any finding indicative of the disease process and only had urothelial cells obscured by any inflammation, blood, mucin. Such samples were excluded from the study. Cases of *low grade urothelial neoplasm* (as reported by The Paris System guidelines) were not found in this study.

This study was designed to evaluate both the diagnostic precision as well as accuracy of urine

cytology as reported by The Paris System, thus, follow up of the cases with histopathologic diagnoses was necessary. After the surgical procedure – either a transurethral resection of urinary bladder tumours (TURBT) or a radical cystectomy, surgical processing of the specimens in the Department of Pathology was done. The specimens were examined grossly and kept for fixation in 10% formalin overnight. Light microscopic examination was done and the histopathological diagnoses of each case was done according to the categories stated by the ISUP/WHO classification system 2016.[5] The correlation between cytology and histopathology was done and considering the latter as the "gold standard", statistical analysis for evaluation of cytology as a diagnostic method was done.

Selection of Thresholds for Reporting

Since more than one diagnostic categories were present in cytology, we used *SHGUC* (Suspicious for High Grade urothelial carcinoma) as the threshold for considering urine cytology as positive, since this threshold appeared to present a scenario closest to that of the clinical follow up of the patients by the urologists. Both the categories- *SHGUC* (Suspicious for High Grade urothelial carcinoma) and *HGUC* (High Grade urothelial carcinoma) had similar cytologic criterion regarding cellular morphology and differed only with respect to gross variations in cellularity, hence these categories were grouped together in this study and considered positive for urine cytology. The other categories under The Paris system, i.e. *NHGUC* (Negative for High Grade urothelial carcinoma), *AUC* (Atypical Urothelial Cells), *LGUN* (Low grade urothelial Neoplasm) clearly denotes the absence of morphologically high grade/ malignant cells, hence, in this study, these were grouped together and considered negative for the test. Hence taking *SHGUC* as the cut off point for positive urine cytology, the diagnostic categories were arranged as shown in Table 1.

Table 1: Diagnostic Categories in Cytology as per selected threshold.

Urine cytology results	Diagnostic categories considered positive for the threshold
Urine cytology test- POSITIVE (i.e. test detected high grade cells)	• Suspicious for high grade carcinoma (SHGUC) • High grade urothelial carcinoma (HGUC)
Urine cytology test NEGATIVE (i.e. test detected NO high grade cells)	• Atypical urothelial cells (AUC) • Negative for high grade urothelial carcinoma (NHGUC) • Low grade urothelial neoplasms (LGUN)

With comparison to the cytology report as positive or negative for high grade urothelial carcinoma, the histological condition was assessed- whether histological high grade morphology was present or absent. The selection of threshold of high grade in histopathology was according to the WHO classification of Urinary Tract Tumours, 2016, as shown in Table 2. Carcinoma in situ, though by definition a high grade lesion was excluded from the category of high grade lesions in this study, as we did not get any case of the same in this study.

Table 2: Diagnostic Categories in Histopathology

Histopathologic results	Diagnostic categories considered positive for the chosen threshold
Positive For High Grade Condition	• Infiltrating urothelial carcinoma • Papillary urothelial carcinoma- high grade
Negative For High Grade Condition	• Papillary urothelial carcinoma- low grade • Papillary urothelial neoplasm of low malignant potential • Inverted papilloma

Results & Analysis

In all the 72 cases, urine cytology findings were reported as per The Paris System and the number of cases in each category was noted, as depicted in Table 3, the graphical representation of which is shown in a bar diagram for frequency of cases in each category-(Figure 1). Overall, cases of urine cytology –POSITIVE (i.e. high grade detected) constituted 66.67% (48/ 72) of all urine cytologic cases. Of all positive cytologic cases, 58.33% (28/

48) comprised of High Grade Urothelial carcinoma (HGUC) while the rest, i.e. 41.66% (20/ 48) comprised of Suspicious for High Grade Urothelial carcinoma (SHGUC). Urine cytology – NEGATIVE (i.e. high grade not noted) constituted 33.33% (24/ 72) of the urine cytologic cases. Of all negative cases, 41.67% (10/ 24) comprised of Negative for High Grade Urothelial carcinoma (NHGUC) and the rest i.e. 58.33% (14/ 24) comprised of Atypical Urothelial Cells (AUC).

The histopathological diagnoses of these 72 cases were done and the number of cases in each diagnostic category was noted, as depicted in (Table 4 & Figure 2). Overall, cases with high grade condition POSITIVE constituted 63.89% (46/ 72) of all the cases. Of the positive for high grade conditions, 91.3% (42/ 46) cases comprised of a histopathological diagnoses of Invasive urothelial carcinoma, while the rest i.e. 8.7% (4/ 46) cases comprised of Papillary Urothelial carcinoma- high grade. Cases with high grade condition NEGATIVE constituted 36.11% (26/ 72) of all the cases. Of these negative conditions, 7.7% (2/ 26) cases comprised of inverted papilloma, 38.46% (10/ 26) comprised of papillary urothelial Neoplasm of low malignant Potential (PUNLMP) and 53.84% (14/26) cases comprised of low grade papillary urothelial carcinoma.

For correlation between cytology and histopathology, data was analyzed as per the diagnostic thresholds selected. Accordingly, the histopathologic findings in the cases labelled as URINE CYTOLOGY POSITIVE (*SHGUC* & *HGUC)* (48/72),included histopathologically diagnosed Invasive Urothelial Carcinoma comprising 79.16% (38/48), High Grade papillary Urothelial neoplasm (HGPUN) comprising 4.17% (2/48), Low Grade papillary Urothelial neoplasm (LGPUN)

Table 3: Number of cases and their respective percentages in the cytologic categories as per the Paris System

Urine Cytology Categories (n=72)	No. Of Cases	Percentage Of Total
Negative for high grade	10	13.89%
Atypical urothelial cells	14	19.44%
Suspicious for high grade	20	27.78%
High grade	28	38.89%

Table 4: Number of cases and their respective percentages in the histopathologic categories as per WHO classification of Urinary tract tumours (2016)

Histopathologic Diagnoses (n=72)	No. Of Cases	Percentage
Inverted papilloma	2	2.78%
PUNLMP	10	13.89%
Low grade papillary urothelial carcinoma	14	19.44%
High grade papillary urothelial carcinoma	4	5.56%
Invasive urothelial carcinoma	42	58.33%

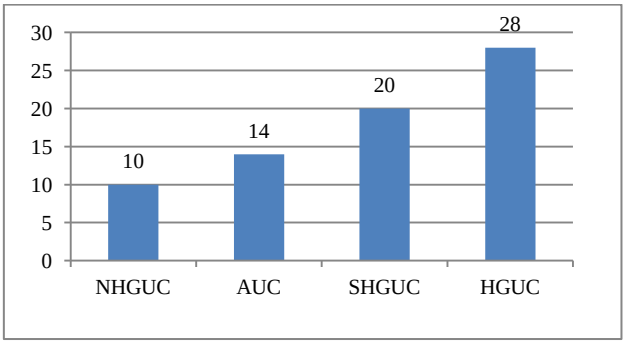


Figure 1: Bar diagram showing frequencies of cases according to cytologic classification of Paris system

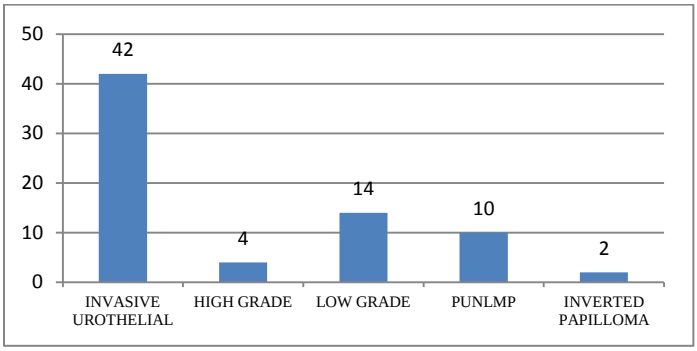
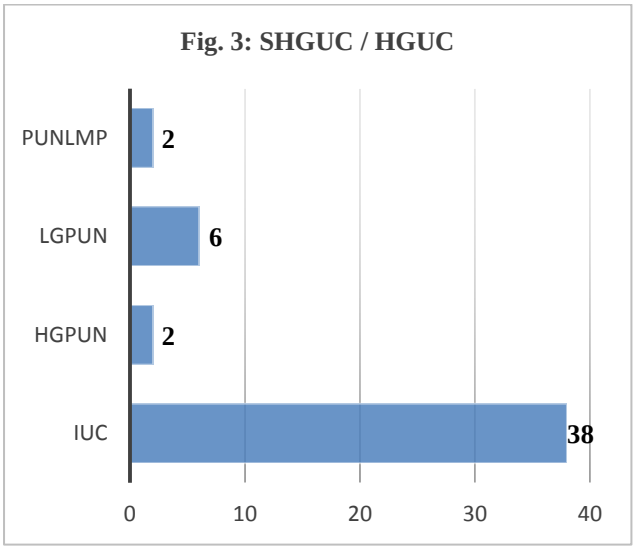


Figure 2: Bar diagram showing frequency of cases according to Histopathological classification (WHO)

Cases labelled as URINE CYTOLOGY NEGATIVE*(NHGUC & AUC)* (24/72) included histopathologically diagnosed Inverted papilloma and High Grade Papillary Urothelial carcinoma each comprising 8.33% (2/24), PUNLMP and Low grade papillary urothelial carcinoma each comprising 33.33% (8/24) and Invasive carcinoma comprising 16.68% (4/24) of the cases.(Figure 4)



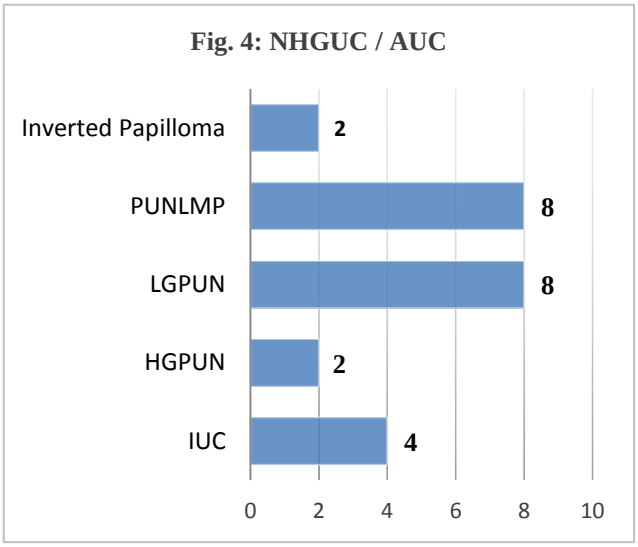


Figure 3: The histopathological findings in cases of positive urine cytology along with the number of cases in each histopathology category.

Figure 4: The histopathological findings in negative urine cytology. The figures clearly denote that the cases with positive cytology truly have a high grade lesion as confirmed by histopathology. In other words, urine cytology can detect most of the high grade lesions

Statistical Analysis

Taking *SHGUC* as the cut off for positivity in urine cytology, appropriate statistical analysis was done for qualitative data and Chi square test was done including the cytology findings being correlated with the gold standard test of histopathology (Table 5).

Table 5: A 2x2 Chi Square table showing the histologic correlations in urine cytology positive and negative cases.

	HISTOLOGY	
CYTOLOGY	POSITIVE FOR HIGH GRADE CONDITION (invasive carcinoma / papillary carcinoma - high grade)	NEGATIVE FOR HIGH GRADE CONDITION (papillary carcinoma - low grade/PUNLMP/ inverted papilloma)
POSITIVE (HGUC / SHGUC)	40	8
NEGATIVE (NHGUC / AUC)	6	18

Sensitivity and specificity

The sensitivity of urine cytology in accurately diagnosing the high grade conditions is calculated to be 86.95%. The specificity is calculated to be 69.23%.

The Yates corrected *p* value of urine cytology is 0.0000571.

The above statistical findings denote urine cytology in the light of the Paris system as a significant test.

Diagnostic accuracy

The positive predictive value of urine cytology was calculated to be 83.33% and negative predictive value was 75%, both denoting the diagnostic accuracy of the test. The diagnostic odds ratio was calculated to be 15, thus denoting the usefulness or effectiveness of the test as well as the test being of better performance.

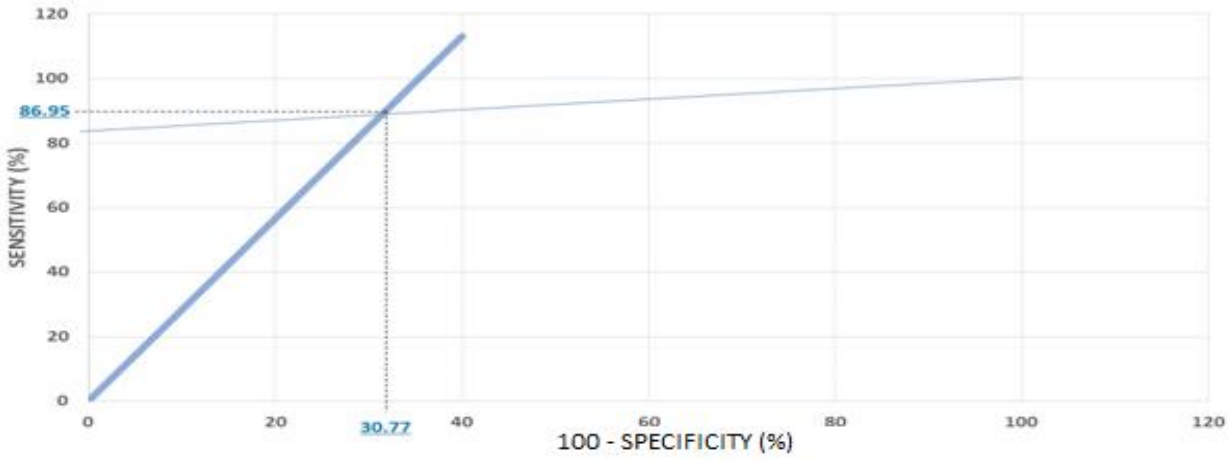


Figure 5: A ROC curve for qualitative data is also plotted on a graph with (100- specificity) on X-axis and sensitivity on Y-axis. The co-ordinate marked in the graph was plotted according to the study results. Slope of the line (bold) joining this co ordinate with the origin gives the likelihood ratio for a positive result. Slope of the line (thin) joining this co ordinate with the highest point, gives the likelihood ratio for negative results.

Although clinicians are well familiar with predictive values, these measures significantly depend on the prevalence of the disease in the population tested. For this purpose, another measure called Likelihood Ratio (LR) was also calculated which is independent of prevalence.[6,7] The Likelihood ratio for a positive result (LR+) was calculated to be 2.82, which denotes a fair probability of positive urine cytology to occur in subjects with high grade urothelial carcinoma than in the rest. Thus urine cytology as reported by the Paris System is a fair test for ruling-in diagnosis. The likelihood ratio for negative result (LR-) was calculated to be 0.18, which denotes less probability of negative urine cytology to occur in subjects with a high grade lesion, in comparison with those not having any high grade lesion. This low value indicates the usefulness of urine cytology for ruling–out diagnosis as well. Figure 5 shows both the likelihood ratio for a positive result (LR+) (bold line) and the likelihood ratio for a negative result (LR-) (thin line) plotted on a ROC curve as done for qualitative data. In the graph, the co ordinate was plotted according to the study results of sensitivity and specificity. For (LR+), the bold line was drawn, joining the test co ordinate with the origin (0,0) The slope of the line gives the value of (LR+) i.e., 2.82. For (LR-), the thin line was drawn, joining the test co ordinate with (100, 100).

In order to confirm the validity or accuracy of the study using *SHGUC* as the cut off point for positive urine cytology, the two other cut off points – *AUC & HGUC* were also applied. The respective sensitivity and specificity using these cut offs were calculated in a similar way and the values were tabulated as shown in Table 6. These values were plotted on the ROC curve. (Figure 6). The distance of the plotted co ordinates along with the values of sensitivity and specificity determines the best credibility as a cut off for the test. The graph clearly shows that using *SHGUC* as the cut off for positive urine cytology provides the best combination of sensitivity and specificity in comparison with *AUC* or *HGUC*. When *AUC* is used as the cut–off, specificity of the test decreases. On the other hand, when *HGUC* is taken as the cut-off, the specificity increases to 100%, but the sensitivity of the test decreases.

Table 6: the values of sensitivity, specificity and (100- specificity), taking AUC, SHGUC and HGUC as cut off.

Cut-off	Sensitivity (%)	Specificity (%)	(100-Specificity)
AUC	95	30.77	69.23
SHGUC	86.95	69.23	30.77
HGUC	60.87	100	0

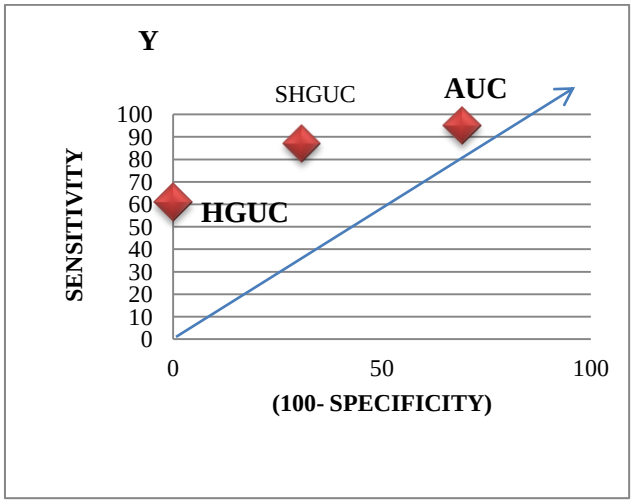
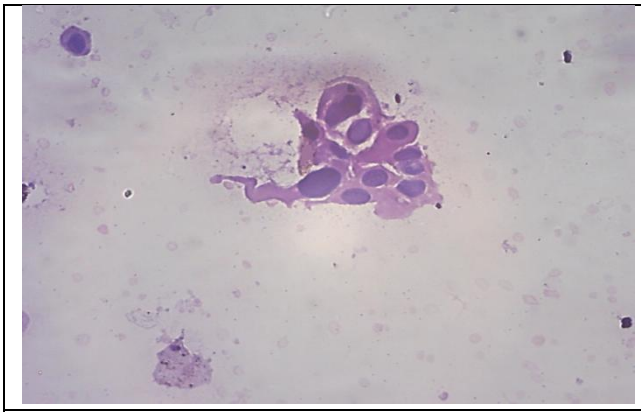


Figure 6: The graph shows the cut offs plotted on a X-Y graph, the one with greater distance from the central line being a better cut off. HGUC also has more distance from the central line than AUC, but the sensitivity of urine cytology decreases greatly, when it is used as the cut-off. Hence SHGUC provides the best cut-off.



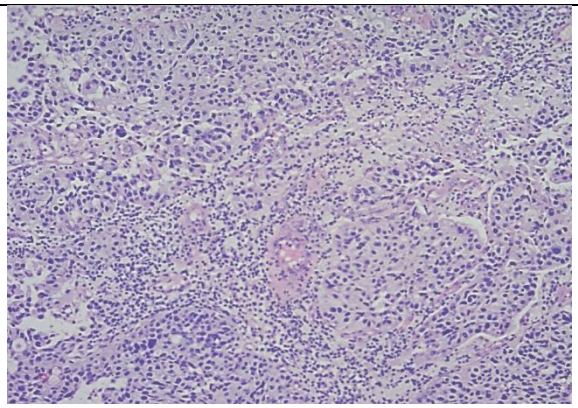
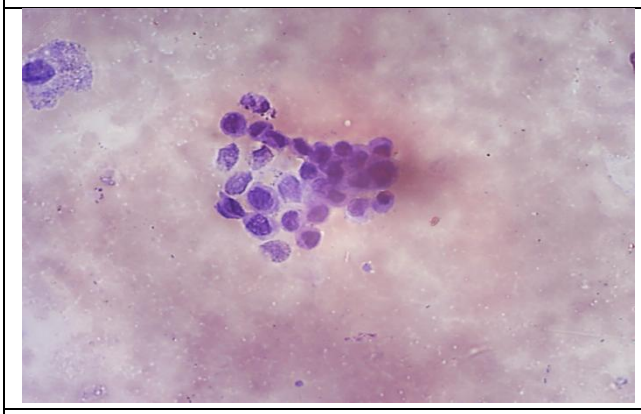


Figure 7: The micrograph on left shows the high power view of urine cytology of one of the cases. The smear shows features of high grade urothelial cells. The count of cells being 5-10, it was designated as a case of Suspicious for High grade urothelial carcinoma. Micrograph on right shows low power view of the histopathology of the same case after a trans urethral resection, characteristic of Invasive Carcinoma.



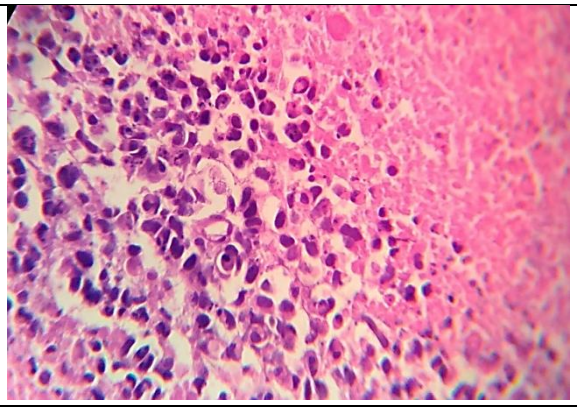


Figure 8: The micrograph on left shows high cellularity of urine cytology of one of the cases showing high grade cells, a case of High Grade Urothelial Carcinoma. Micrograph on right shows the histopathological finding (high power) view, characteristic of Invasive Carcinoma.

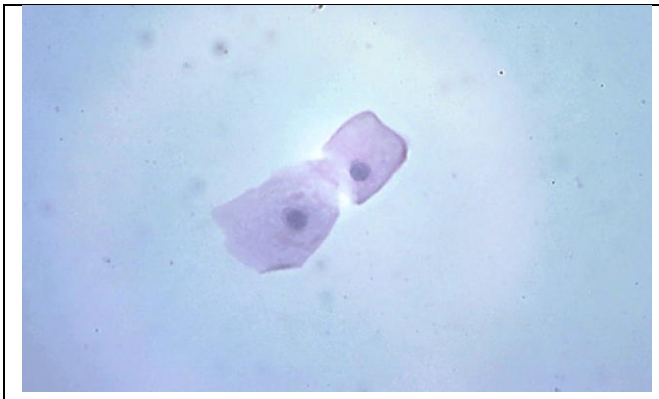
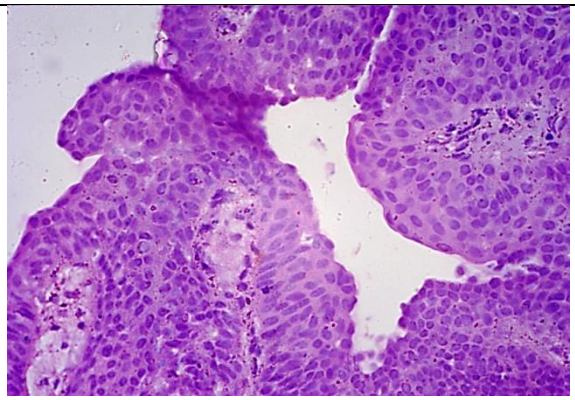



Figure 9: The micrograph on left shows the urine cytology finding of a case, designated as Negative for High Grade Urothelial carcinoma. Micrograph on right shows the histopathological finding of the TURBT specimen of the case, characteristic of Papillary Urothelial Neoplasm of Low malignant potential.(PUNLMP).

Discussion

Urine, among other body fluids is a heterogeneous mixture, containing non solute particles, crystals, microorganisms. Also, it contains cells shed from the entire urinary tract along with chemical substances like urea, creatinine, that markedly affect the osmolality of urine. Hence, preservation and distribution of cells throughout the urine volume is not uniform.[8] With appropriate, yet simple preservation and cell retrieval methods like centrifugation, the denser cells can be examined. It was stated in The Paris System that a urine sample could be called inadequate only if it did not show any finding indicative of any disease process or if urothelial cells are obscured by lubricants, mucin or blood. Hence, using TPS, urine cytology became an important non invasive technique for screening, diagnosis and follow up of patients with urothelial carcinoma

Several studies were conducted comparing the role of other methods of cytological study like Liquid Based Cytology with that of simple centrifugation technique. No significant differences in sensitivity or specificity were observed between the two methods in cases of high grade carcinoma.[9,10] Though some studies described the superiority of LBC of urine in terms of increased cellular morphology, cellularity and cleaner background, other studies concluded that in simple centrifugation, malignant cells were clumped together and had conserved papillary architecture whereas LBC techniques resulted in the diagnostic cells becoming small, shrunken and dispersed, thus blurring the distinction between normal, reactive, malignant or degenerated cells.[11] This disadvantage of LBC could not be overlooked, since all the diagnostic categories of TPS included study of non degenerated cells only.

TPS has clearly achieved its goal of reducing the rate of "atypical" cells in urine cytology reporting since all reactive changes are included in the category of "negative" urine cytology. Hence, whereas previously, subjects assigned with a diagnosis of "atypical" cells were managed in a way similar to "negative" cases, after the implementation of TPS, the criteria of AUC group becoming clearer, will require such cases being managed by closer follow-up and evaluation. TPS also cleared the distinction between SHGUC & HGUC. While cellular morphological criteria for both the groups were similar the difference between them was clearly stated to be the quantity of cells showing such features. Thus TPS not just clearly defined the conditions to be included in the category of NHGUC, it also clearly stated the distinct features of each borderline categories between negative and malignant. Three dimensional papillary clusters of cells containing fibrovascular cores was stated as the only condition where LGUN could be assigned. None

of the cases in this study was diagnosed with such feature, the probable reason of which could be the study being conducted with voided urine samples which causes cells to be shed more individually than in papillary clusters. Despite this fact, the failure to detect LGUN cannot be called a false negative result in favour of TPS, since TPS very clearly was designed not to detect LGUN, rather to ensure not missing out cases of high grade carcinomas.[(12)]

Figure 7, 8 & 9 show the cytological findings and their corresponding histopathological findings of three of the cases in the study. As seen in the study results, some false negatives were noted in urine cytology, which is expected, as voided urine samples were used which contain tumour cells shed before reaching a diagnosable state. Urothelial cells start degenerating even before exfoliation into urine and continue doing so throughout their exposure in urine, i.e. both prior to and after voiding. Inter-observer variation need to be studied in order to check the reproducibility of the "atypical" category.

The statistical analyses in the study clearly showed an increased rate of detecting HGUC and reduced rate of reporting AUC. The diagnostic accuracy of urine cytology as reported by TPS was clearly high.

Conclusion

Evaluation of the reliability and accuracy of urine cytology as per The Paris System, in correlation with histopathology as the gold standard, was the main objective of this study. The statistical correlations in this study have proved urine cytology as a highly useful test for diagnosing urothelial carcinomas. This is in wide contrast to the accuracy of urine cytology as a diagnostic method, prior to the establishment of The Paris System. For both urologists as well as pathologists, understanding the diagnostic criteria, their clinical implications, and appreciating the limitations of cytopathology in general, is necessary if we are to utilize urine cytology and ancillary tests in a thoughtful and practical manner. The Paris System provides a common framework and has hence, ensured the acceptance and adoption of urine cytology in practical settings by most pathologists and clinicians.

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