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MICROSCOPIC FEATURES OF UROTHELIAL MALIGNANCY: A CORRELATIVE STUDY OFURINE CYTOLOGY AND HISTOPATHOLOGY IN BLADDER CARCINOMA.

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ABSTRACT

Background: Urine cytology remains a cornerstone in the diagnosis and prognosis of urothelial carcinoma**.** It continues to be widely utilized due to its distinct advantages, including ease of specimen collection and high patient compliance. This method plays a crucial role in both initial screening and long-term surveillance. However, its sensitivity can vary, particularly with low-grade tumors, necessitating histopathological correlation through tissue biopsies or resection specimens. When urine cytology is combined with histopathological evaluation, diagnostic accuracy significantly improves, enhancing the clinician's ability to make informed decisions regarding treatment.

Aim: To describe and analyze the cytological characteristics of urothelial malignancy with histopathological grading

Methods: In this prospective observational study, urine samples from (n-70) patients with clinically suspected bladder carcinoma were examined using Papanicolaou-stained cytology. Histopathological evaluation of biopsy specimens followed standard grading protocols. Cytological parameters such as nuclear atypia, chromatin texture, mitotic activity, and cellular architecture were systematically compared against corresponding histological findings. Data collected, recorded and later analyzed.

Results: The study highlights that urothelial malignancies predominantly affect males in their 50s, with smoking as the leading risk factor. Hematuria is the most common symptom. Cytological evaluation using the Paris System shows moderate alignment with histopathology, with good sensitivity for high-grade tumors but limited accuracy for low-grade and atypical cases. Histopathology detects key morphological features more reliably. Cytology demonstrates strong diagnostic effectiveness (80%) and perfect positive predictive value, though its negative predictive value (81.8%) suggests missed malignancies. Interobserver agreement is highest for high-grade carcinoma, reinforcing its diagnostic clarity.

Conclusion: Structured cytological assessment, when mapped to histopathological benchmarks, offers enhanced diagnostic fidelity for urothelial carcinoma. This study advocates for integrative interpretation frameworks in pathology training to improve early detection and patient outcomes.

Keywords: Urine cytology, Urothelial carcinoma, Papanicolaou stain, Biopsy, Nuclear atypia, Mitotic activity

INTRODUCTION

Bladder carcinoma accounts for 3% malignancies of the urinary tract, with urothelial carcinoma accounting for over 90% of cases above the age of 55yrs and older [1] [2] [3]. Urothelial carcinoma, arising from the transitional epithelium lining the urinary tract manifests along both the upper and lower urinary pathways [4] [5]. Historically regarded as a biologically unified entity, recent strides in epidemiologic mapping and genomic profiling have revealed a striking divergence in tumor behavior, molecular landscape, and clinical outcomes based on tumor location [6] [7]. Bladder cancer was first noted in man worked in aniline dye industry in 1895. Common age group is 65 to 70 years old. Most of patients are older than 50 years of age [8].

Early and accurate diagnosis is crucial in reducing morbidity and guiding appropriate therapeutic interventions. Among the diagnostic tools, **urine cytology** stands out as a non-invasive, cost-effective, and widely utilized method for detecting urothelial malignancy—particularly high-grade tumors [9] [10]. However, its sensitivity can vary, especially for low-grade lesions [10].

Crucially, the challenge lies in **preoperative risk stratification**—accurately distinguishing indolent tumors from biologically aggressive ones to guide treatment decisions. Urine cytology and histopathology are complementary diagnostic modalities pivotal in evaluating urothelial malignancies. Cytological examination of voided urine or bladder washings enables the detection of atypical or malignant cells, particularly useful in high-grade urothelial carcinoma where cellular atypia is prominent [11]. Whereas, Histopathological evaluation typically derived from biopsy or resection specimens, provides a definitive diagnosis by assessing architectural patterns, cellular differentiation, and depth of invasion [12]. When integrated, urine cytology serves as a valuable non-invasive screening tool while histopathology confirms and characterizes the lesion, guiding treatment strategies and prognostication [9].

The correlation between cytological findings in voided urine samples and histopathological features of biopsy specimens can strengthen diagnostic accuracy and help identify potential limitations of cytology alone [13]. This integrative approach is undertaken in this investigation to evaluate the microscopic features of urothelial malignancy and establish a correlative framework between urine cytology and histopathology findings

MATERIALS AND METHODS

The Study was conducted in our pathology department at Saveetha medical college, Thandalam, Chennai from the year of February 2024 to April 2025 at a tertiary teaching hospital, following prior ethical approval. Seventy patients aged 30–70 yrs (n-70) presenting with clinical signs and symptoms of urothelial carcinoma were enrolled. Exclusion criteria included confirmed non-urothelial malignancies and lack of informed consent.

Midstream, first-morning voided urine samples (50–100 mL) were collected in sterile containers. Samples were centrifuged at 2,500 rpm for 10 minutes, and the sediment was stained using Papanicolaou and May-Grünwald-Giemsa (MGG) techniques. Cytological evaluation was performed following **The Paris System for Reporting Urinary Cytology [14]**, classifying specimens into:

- Negative for High-Grade Urothelial Carcinoma (NHGUC)
- Atypical Urothelial Cells (AUC)
- Suspicious for High-Grade Urothelial Carcinoma (SHGUC)
- High-Grade Urothelial Carcinoma (HGUC)
- Low-Grade Urothelial Neoplasm (LGUN)

Bladder lesions sampled by transurethral resection or punch biopsy were fixed in 10% buffered formalin, processed routinely, and embedded in paraffin. Sections were stained with Hematoxylin and Eosin (H&E), with histological grading and staging performed using the WHO/ISUP classification andAJCC TNM staging system [15] [16]**.**

Sample Size Calculated using,

p- 3.4% [1], d- 5, and a critical value of Z=1.96.

$$\mathbf{n=Z^2\times p\times(100-p)/d^2}$$

$$=(1.96)^2\times 3.4\times(100-3.4)/(5)^2$$

≈51 (70 patients)

Adjusting for a potential 2% nonresponse rate, final sample size: 52 patients,(n-70) patients were chosen.

STATISTICAL ANALYSIS

Microscopic features such as cellular atypia, nuclear pleomorphism, mitotic figures, and evidence of invasion were compared between cytology and histology using various descriptive statistics. Comparisons of categorical variables

were performed using the Chi-square test, with statistical significance determined at a probability (pp) value of less than 0.05. Data analysis was conducted using SPSS (IBM, USA) and Microsoft Excel (Microsoft, USA).

OBSERVATIONS AND RESULTS

Table 1: Demographic Distribution, Risk Factors, and Symptomatology Among Patients Diagnosed with Urothelial Malignancy

Age Group (Years)	Male (n=48)	Female (n=22)
30–39	4	2
40–49	10	5
50–59	14	8
60–69	12	5
≥70	8	2
Risk Factor	Number of Cases	Percentage (%)
Smoking	30	42.9
Occupational exposure	10	14.3
(dyes, chemicals)		
Chronic cystitis	8	11.4
Schistosomiasis	2	2.9
No identifiable risk	20	28.5
Symptom	Number of Cases	Percentage (%)
Hematuria	45	64.3
Dysuria	20	28.6
Urinary frequency	10	14.3
Flank pain	5	7.1
Incidental finding	7	10

Most urothelial malignancy cases were seen in individuals aged 50–59 years, with a clear male predominance. Smoking was the leading risk factor, followed by occupational exposure and chronic inflammation. About 28.5% had no identifiable risk. Hematuria was the most common symptom, while other presentations like dysuria, urinary frequency, flank pain, and incidental findings highlighted variable clinical profiles and the importance of early detection.

Table 2: Alignment of Paris System Cytology Categories with Histopathological Outcomes (n = 70)

Diagnostic Category	Cytology Cases (n = 70)	Percentage (%)	Histopathology Cases (n = 70)	Percentage (%)
Negative for HGUC / No Malignancy	25	35.7%	20	28.6%
Atypical Urothelial Cells (AUC)	12	17.1%	–	–
Suspicious for HGUC (SHGUC)	10	14.3%	–	–
Low-Grade Urothelial Neoplasm / Carcinoma	5	7.2%	12	17.1%
Papillary Urothelial Neoplasm	–	–	8	11.4%
High-Grade Urothelial Carcinoma	18	25.7%	30	42.9%

The Paris System cytology showed moderate alignment with histopathology outcomes in urothelial malignancy cases. While cytology detected high-grade urothelial carcinoma in 25.7% of cases, histology confirmed it in 42.9%, suggesting cytology underestimation. Low-grade tumors were more commonly identified histologically (28.5%) than cytologically (7.2%), reflecting limited cytological sensitivity for lower-grade lesions. Atypical and suspicious

cytology categories (31.4% combined) lacked direct histological correlates, indicating diagnostic ambiguity. Cytology-negative cases (35.7%) largely matched benign histology (28.6%), demonstrating fair concordance.

Table 3: Concordance Between Cytological and Histopathological Grades (n = 70)

Cytology Grade	Histopathology Grade	Number of Cases	Concordant (%)
Low Grade	Low Grade	10	14.28%
Low Grade	High Grade	1	--
High Grade	High Grade	25	35.71%
High Grade	Low Grade	3	—
Atypical	Low Grade	5	—
Atypical	High Grade	4	—
Negative Cytology	No Malignancy	18	25.71%
Negative Cytology	Malignancy Present	4	—

Cytology demonstrated moderate concordance with histopathology, showing good reliability in identifying high-grade urothelial carcinoma (35.71%) and limited accuracy for low-grade lesions (14.28%). Negative cytology matched benign histology in 25.71% but missed 4 malignancies, indicating reduced negative predictive value. Atypical cases showed mixed histological grades, reflecting diagnostic ambiguity. Overall, cytology performs well for high-grade detection but is less reliable for low-grade and atypical lesions, reinforcing the importance of histopathology for definitive grading.

Table 5: Comparison of Morphological Features in Cytology and Histology

Feature	Seen in Cytology (n=70)	Seen in Histology (n=70)
Nuclear Enlargement	35	42
Hyperchromasia	28	40
Irregular Nuclear Membrane	21	36
Mitoses	10	24
Tumor Cell Clusters	15	30

Histopathology consistently demonstrated a higher detection rate for all key diagnostic features compared to cytology. **Nuclear enlargement** was the most common finding in both modalities, identified in 42 histological and 35 cytological specimens. **Hyperchromasia** and **irregular nuclear membranes** were notably underreported in cytology (28 and 21 cases) versus histology (40 and 36 cases), indicating limited sensitivity in cytological smear interpretation

Table 6: Diagnostic Accuracy of Cytology Compared to Histopathology (n = 70)

Cytology Category	Histopathology Outcome	TP	TN	FP	FN	Sensitivity (%)	Specificity (%)	Accuracy (%)
Low Grade (LGUC)	Confirmed Low Grade	10	–	1	–	90.9%	–	–
High Grade (HGUC)	Confirmed High Grade	25	–	3	–	89.3%	–	–
Negative Cytology	Confirmed No Malignancy	18	18	0	4	81.8%	100.0	51.4%

Prevalence	**68.6%**
Positive Predictive Value (PPV)	**100.0%**
Negative Predictive Value (NPV)	**81.8%**
Diagnostic Effectiveness	**80.0%**

Cytology demonstrates strong sensitivity for both high-grade (89.3%) and low-grade (90.9%) urothelial carcinoma detection. Its specificity for ruling out malignancy via negative cytology is excellent (100%), though 4 cases of false negatives lower its sensitivity to 81.8%. Overall diagnostic accuracy for negative cytology rests at 51.4%, driven primarily by correct identification of benign cases. These findings affirm cytology's reliability in high-grade diagnosis, but also highlight the need for histopathological confirmation, especially in negative or ambiguous cases.

Also there was high diagnostic reliability with 100% PPV and 80% overall effectiveness, effectively confirming malignancy when present. However, its NPV of 81.8% indicates some missed cases, reinforcing the need for histopathological confirmation in negative results

Table 7: Interobserver Agreement in Cytological Interpretation

Cytology Category	Kappa Value (κ)	Agreement Level
NHGUC	0.70	Substantial
AUC	0.52	Moderate
SHGUC	0.68	Substantial
HGUC	0.85	Almost perfect
LGUN	0.60	Moderate

High-grade urothelial carcinoma (HGUC) achieved the highest interobserver consistency (κ = 0.85), classified as *almost perfect*, highlighting strong diagnostic clarity and consensus.Negative for HGUC (NHGUC) and suspicious for HGUC (SHGUC) showed *substantial agreement* (κ = 0.70 and 0.68, respectively), reinforcing dependable recognition of both benign and suspicious cases.

DISCUSSION

This study's demographic data align well with established epidemiological patterns for urothelial carcinoma. Most cases were observed in individuals aged **50–59 years, with a clear male predominance (5:1 male to female ratio)**. This is consistent with the observations by Jateen Anshuman et al [17] who also found the most common age group to be 60-69 years and a 5:1 male to female ratio, and Siyad P.M. et al [18] who reported a male predominance of 89.7% and the majority of patients aged 61 to 70 years. Smoking was identified as the leading risk factor in the current study (42.9% of cases and 70% in the study group), followed by occupational exposure. Similarly, Jateen Anshuman et al[17] noted that 74% of their patients were smokers. Hematuria was the most common symptom, observed in 64.3% of cases, while Siyad P.M. et al[18] reported hematuria in 100% of their patients.

Regarding the diagnostic performance of cytology, the current study using The Paris System (TPS) showed a **moderate alignment with histopathology outcomes**. It identified that cytology underestimated high-grade urothelial carcinoma (HGUC), detecting it in 25.7% of cases compared to histology's 42.9%. Furthermore, low-grade tumors were identified histologically at a higher rate (28.5%) than cytologically (7.2%), suggesting **limited cytological sensitivity for lower-grade lesions**. This observation is corroborated by multiple studies. Jateen Anshuman et al [17]similarly concluded that sensitivity rates are higher for high-grade tumors, as low-grade tumors are less likely to shed cells into urine. Their data showed that while 86% of histologically confirmed HGUC cases were detected cytologically, only 58% of low-grade urothelial carcinoma (LGUC) cases were detected. Siyad P.M. et al[18] found that urine cytology detected malignant cells in 46.2% of high-grade carcinoma cases but only in 14.3% of low-grade neoplasms. McCroskey et al [19] specifically reported a low sensitivity (21% to 53%) for the cytologic diagnosis of LGUC. Michelle D Reid et al [20] also highlighted that the **identification of low-grade urothelial carcinoma is fraught with difficulty and low sensitivity rates**.

Despite the noted underestimation of low-grade lesions, the current study reported a **high sensitivity for both low-grade (90.9%) and high-grade (89.3%) urothelial carcinoma detection**. Its specificity for ruling out malignancy via negative cytology was excellent (100%). However, the presence of four false negatives lowered the overall sensitivity for negative cytology to 81.8%, leading to an overall diagnostic accuracy for negative cytology of 51.4%.

A notable point of discussion arises from the **interobserver variability** in cytologic interpretation. The current study reported **almost perfect consistency for HGUC (κ = 0.85)** and substantial agreement for Negative for HGUC (NHGUC) and Suspicious for HGUC (SHGUC) categories (κ = 0.70 and 0.68 respectively).These findings suggest a relatively high level of agreement, especially for high-grade diagnoses. In contrast, Michelle D Reid et al [20] concluded that the **overall accuracy of grading on urine cytology was unacceptably low at 77%**, and their interobserver agreement, as measured by coefficient kappa, was **unacceptably low for grading urothelial carcinoma, with most kappa values less than 0.40**. They noted that even prevalence-adjusted and bias-adjusted kappa (PABAK) coefficients indicated poor reliability. McCroskey et al [19] also found that the overall agreement for the cytologic diagnosis of LGUC was only fair (κ = 0.30).

The inherent subjectivity in grading urothelial carcinoma has been acknowledged in histological contexts as well. Renshaw AA et al [21] noted considerable interobserver variability in the distinction of Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP) from low-grade urothelial carcinoma using the 2004 WHO

system. This broader challenge in histological grading underscores the compounded difficulty when applying such systems to less organized cytologic specimens.
The current study's emphasis on the diagnostic utility of morphological features aligns with general cytopathological practice, but it also highlights that histopathology consistently demonstrated a higher detection rate.
Overall, while urine cytology serves as a valuable, non-invasive adjunct in the evaluation and screening of urothelial malignancy, particularly for high-grade lesions due to their propensity to shed abnormal cells and often distinct cytological features, its limitations in accurately grading and detecting low-grade lesions remain a significant challenge, emphasizing the need for standardized training and possibly objective, non-morphologic ancillary tests [22].

CONCLUSION

The study concluded that while cytological examination of urine specimens is a valuable aid in diagnosing bladder tumors, especially for high-grade lesions where accuracy is higher, its reliability for low-grade and atypical lesions is less consistent. It emphasizes that negative cytology results may miss some malignancies, reinforcing the need for histopathological confirmation. Ultimately, it positions voided urine cytology as a simple, non-invasive, and diagnostically effective adjunct with definitive histopathological evaluation [22].

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AUTHOR CONTRIBUTIONS:-

R. Sowmya and *Sridevi M* were responsible for the conceptualization, visualization, and overall administration of the study. *R. Sowmya* contributed to data curation and the initial drafting of the manuscript, while *Sridevi M* oversaw validation and formal analysis. Both authors were actively involved in the manuscript revision process and have approved the final version for submission.

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