Original Article

Diagnostic Evaluation of Urine Cytology in Detection of Urothelial Carcinoma

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Abstract

Background: Bladder carcinoma is the tenth most common carcinoma worldwide, representing three percent of global cancer diagnoses. Urine cytology is an important tool for urothelial carcinoma screening.

Materials and Methods: A cross-sectional study was carried out in two hundred seventy-five cases. Urine samples were collected and processed according to the standard procedure and stained with May-Grünwald Giemsa (MGG) and Papanicolaou (PAP) stain, and reported as per The Paris System. The results were then correlated with histopathological findings of transurethral resection of bladder tumor chips. According to the findings observed, a relevant statistical test was applied.

Results: According to The Paris System of reporting, the distribution was as follows: High-grade urothelial carcinoma – seven percent; Suspicious for high-grade urothelial carcinoma – fourteen percent; Atypical urothelial cells – nine percent; and Negative for high-grade urothelial carcinoma – seventy percent. Out of two hundred seventy-five cases of urine cytology, histopathology was possible in forty-five cases, of which forty-two cases were positive for urothelial carcinoma. The sensitivity of urine cytology for detection of urothelial carcinoma is seventy-seven percent, specificity is one hundred percent, and diagnostic accuracy is seventy-eight percent. Male-to-female ratio was 3:1.

Conclusion: Urine cytology has low sensitivity but high specificity for detection of urothelial carcinoma. Accurate diagnosis of urothelial carcinoma can be made by combination of clinical information, urine cytological findings, and histopathological findings.

Keywords:

Urine cytology, Bladder carcinoma, High-grade urothelial carcinoma, The Paris System of Reporting.

Introduction

Bladder carcinoma is the tenth most common carcinoma worldwide, and it represents three percent of all carcinoma [1]. GLOBOCAN 2022 reported approximately 614,298 new cases and 220,596 deaths. The global age-standardized incidence rate was 5.6 per 100,000 people, with the highest rates recorded in Spain (32.4 per 100,000 men). In India, the age-standardized incidence rate for bladder cancer was 2.5 per 100,000, with 17,667 new cases and 12,492 deaths documented in 2022 [2]. In the urinary system, urothelial carcinoma ranks ninth among malignant neoplasms [3]. The male-to-female ratio is 4:1 [4]. The standard

diagnosis is usually made by cystoscopy with tissue biopsy and histopathological examination, but such a diagnostic method is invasive and inconvenient in routine clinical practice. Therefore, urine cytology becomes increasingly useful for urothelial cancer screening, especially in high-risk patients [5]. A standardized, evidence-based, and sufficiently detailed method for reporting urine cytology, the Paris System (TPS), was released as an international reporting system in 2016 [6]. It maintains the standard performance of urine cytology by reducing unnecessary indeterminate diagnoses in detecting high-grade urothelial carcinoma [7]. Cytological diagnosis of a urine specimen is a simple, less expensive, and safe method that may uncover a hidden urothelial carcinoma [8]. It complements but does not replace cystoscopy and histopathological confirmation. Urine cytology is usually used as a screening tool for the detection and follow-up of symptomatic patients, patients with previous urinary tract neoplasms, and detection of cancer in high-risk patients (e.g., those exposed to industrial chemicals and metals, cigarette smokers) [9].

Aim: To evaluate the diagnostic accuracy of urine cytology for detection of urothelial carcinoma as per the Paris System and its histopathological correlation.

Materials and Methods

A hospital-based cross-sectional study was conducted in the Department of Pathology, Gauhati Medical College and Hospital, Assam. Two hundred and seventy-five cases were part of the study from October 2022 to September 2023. Urine cytology detects abnormal cells in urine for diagnosing malignancies like urothelial carcinoma. The process begins with sample collection through voided urine (preferably midstream), or catheterized urine sample (from the catheter, not from the urine bag) in a clean container. The collected urine is then processed by centrifugation at 1500 rpm for 10 minutes, after which the supernatant is discarded and smears are prepared from the sediments. Fixation is immediately performed using air drying for Romanowsky stain and alcohol-based fixation for Papanicolaou stain.

Papanicolaou stain has the following excellent properties: nuclear details seen, cytoplasmic differentiation. Dyes used in Papanicolaou stains are: Harris Hematoxylin, Orange G, and Eosin Azure. Basic steps in Papanicolaou stains are as follows: Rehydration in graded concentration of alcohol (90%, 70%, 50%) – 2 minutes each. Running tap water – 10 minutes. Nuclear staining by hematoxylin – 15 minutes. One percent acid alcohol – 1 to 2 dips. Running tap water – 10 minutes. One percent ammonia water – 1 to 2 minutes. Running tap water – 10 minutes. Dehydration by absolute alcohol (50%, 70%, 90%) – 2 minutes each. Orange G-6 – 2 minutes. Wash with absolute alcohol. Eosin Azure 36 – 2 to 3 minutes. Clearing by xylene. Mounting by DPX mounting medium.

May Grunwald Giemsa is a Romanowsky's stain that provides excellent cytoplasmic detail. Air-dried smears are refixed in methanol for 5 minutes and stained as follows: May Grunwald solution – 5 minutes. Running water – 1 minute. Giemsa stain – 15 minutes. Running water – 1 minute. Air drying

Under low power, all smears were screened. The final diagnosis was given after examination under high power. The cases were classified as per the Paris System into different categories like: Negative for High Grade Urothelial Carcinoma (NHGUC), Atypical Urothelial Cell (AUS), Low-Grade Urothelial Neoplasm (LGUC), Suspicious for High Grade Urothelial Carcinoma (SHGUC), and High-Grade Urothelial Carcinoma (HGUC).

Every urine cytological sample, whether voided or catheterized, was included in the study if there was a clinical suspicion of urothelial neoplasm of the urinary bladder, covering all genders and age groups. Unsatisfactory and inadequate samples were excluded from this study. Histopathology was possible for forty-five cases. Paraffin blocks were made, stained with hematoxylin

and eosin, and estimated histopathologically to identify urothelial carcinoma according to WHO standards.

Histopathological categories according to WHO Classification [2022] are: Noninvasive urothelial neoplasms; Urothelial Papilloma; Inverted Urothelial Papilloma; Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP); Noninvasive Papillary Urothelial Carcinoma, Low Grade; Noninvasive Papillary Urothelial Carcinoma, High Grade; Invasive Urothelial Carcinoma.

All data were analyzed in MS Excel sheet. The sensitivity, specificity, and diagnostic accuracy of urine cytology were calculated with $p < .05$ set as significant. Ethical approval from the Hospital's Ethical Committee [No. MC/190/2007/pt-II/Sept.2022/46] was in place throughout the study.

Results

A total of two hundred seventy-five cases were analyzed, of which seventy-five percent (two hundred and seven) cases were male and twenty-five percent (sixty-eight) cases were female. The maximum number of cases was between sixty-one to seventy years of age, i.e., thirty percent (eighty-four cases), and the most common clinical presentation was hematuria, i.e., thirty-four percent (one hundred and four) of the cases. In cytology, seventy percent (one hundred and eighty-six cases) of the cases were diagnosed with NHGUC, which is mostly present in patients between fifty-one and seventy years of age, and in histopathology, sixty percent (twenty-seven) of the cases were diagnosed with HGUC. After analyzing all the data, the sensitivity, specificity, and diagnostic accuracy of the present study are found to be seventy-seven percent, one hundred percent, and seventy-eight percent, respectively.

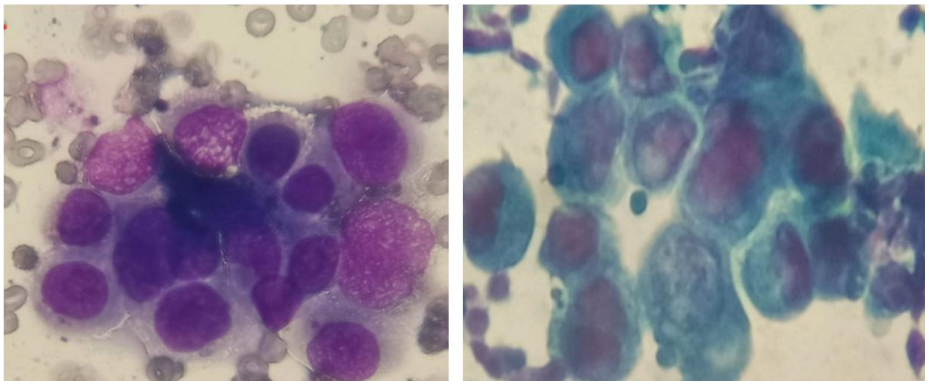

Figure 1: High-grade urothelial carcinoma (HGUC), 40× view, MGG and PAP stain.

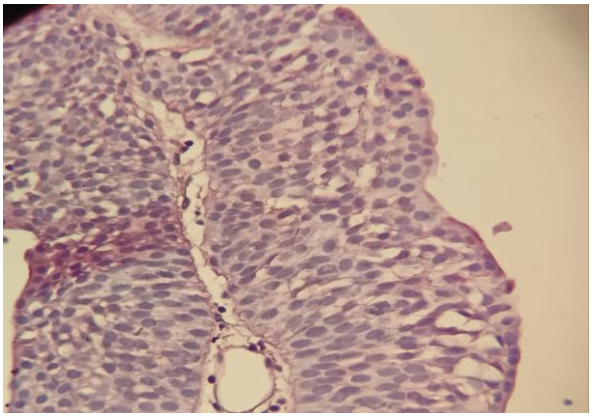

Figure 2: Low-grade urothelial carcinoma (LGUC), 40× view, H&E stain.

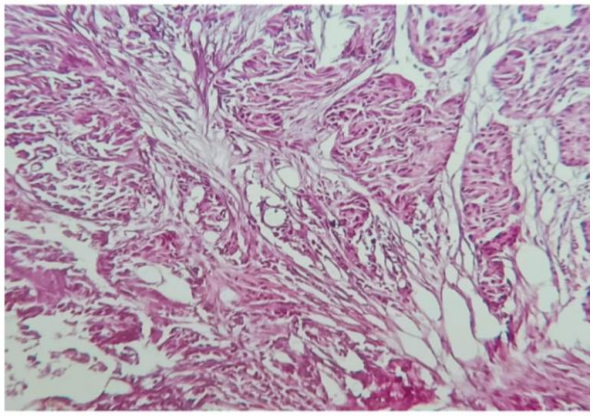

Figure 3: Invasive urothelial carcinoma, 40× view, H&E stain.

Table 1: Distribution of cytological diagnosis as per the Paris System

CYTOLOGY	FREQUENCY	PERCENTAGE
NHGUC	186	70%
AUC	23	**09%**
SHGUC	38	14%
HGUC	19	07%

The most common cytological diagnosis is NHGUC, in seventy percent (one hundred eighty-six) cases.

Table 2: Distribution of cases according to Histology and cytology

	HPE Positive	HPE Negative
Cytology Positive	42(TP)	0(FP)
Cytology Negative	12(FN)	03(TN)

Sensitivity: seventy seven percent, Specificity: hundred percent, Diagnostic Accuracy: seventy eight percent.

Discussion

George Papanicolaou was the pioneer in cytopathology, and he popularized urine cytology in the 1940s to detect and follow up patients with bladder neoplasm [10]. Bladder cancer is the tenth most common cancer in the world, the 17th most common cancer in India, and it ranks 19th in mortality across the Indian population [11]. There are sex and gender disparities in bladder carcinoma incidence, with a higher male preponderance, i.e., males are at four times higher risk than females [12]. For males, the median age of diagnosis is approximately sixty-nine years old, whereas for females, it is approximately seventy-one years old [13]. The multifactorial pathogenesis of bladder carcinoma is mainly due to either intrinsic genetic factors or external environmental factors [14]. Several risk factors associated with the occurrence of bladder cancer include genetic and hereditary factors, smoking and tobacco use, increased body mass index, occupational exposure to certain chemicals and dyes, medical conditions such as chronic cystitis, and infectious diseases such as schistosomiasis [15].

The commonest predictor of bladder cancer is hematuria. Abdominal pain, lower extremity edema, and flank pain are some rare symptoms that may occur with advanced stages of bladder cancer [16]. Urine cytology plays an important role in early bladder

cancer diagnosis by evaluating suspicious urine cytology results in conjunction with other investigations [17]. The reporting system for urine cytology has evolved over a period of time based on changes in the histopathological classification of the World Health Organization. Urine cytology is a simple, quick, and less expensive investigation for screening and detection of high-grade urothelial carcinoma, and it provides essential and adequate information for physicians to plan for care and further testing to obtain a conclusive diagnosis [18].

The purpose of the Paris System of Reporting is to have the highest positive predictive value for high-grade urothelial carcinoma. Criteria for diagnosis of high-grade urothelial carcinoma are: 5–10 malignant cells for lower tract specimens and >10 cells for upper tract specimens, with cytological findings of a nucleo-cytoplasmic ratio of >0.7, moderate to severe hyperchromasia, irregular nuclear membranes, coarse chromatin, and cellular pleomorphism [19]. A positive urine cytology denotes a urothelial tumor in the urinary tract, but a negative cytology does not rule out the presence of a tumor [20].

In the present study, male:female is 3:1, which is similar to Rai S et al, Chan et al, and Rohilla et al, but according to Chan et al, it is 4:1 (Table 3). Sensitivity, specificity, and diagnostic accuracy of the present study for detection of high-grade urothelial carcinoma in urine cytology are seventy-seven percent, one hundred percent, and seventy-eight percent, respectively. In comparison with the previous studies included in Table 4, sensitivity of the present study is almost similar to Rai S et al, Hasan et al, and Rohilla et al, but higher than Chan et al. Specificity of the present study is similar to Chan et al but higher than Rai S et al, Hasan et al, and Rohilla et al. Diagnostic accuracy of the present study is almost similar to the previous studies.

Table 3: Comparing the male: female of the present study with previous studies.

STUDIES	MALE: FEMALE RATIO
Present study	3:1
Hasan et al[3]	4:1
Sharada Rai et al [5]	3:1
Rohilla M et al [21]	3.7:1
Chan E et al [22]	3:1

Table 4: Comparing the statistical parameters of the present study that of previous studies.

VARIABLES	PRESENT STUDY	SHARADA RAI et al [5]	HASAN et al [3]	ROHILLA et al [21]	CHAN E et al [22]
Sensitivity	77.7%	83.3%	83.3%	70.5%	62.2%
Specificity	100%	89.4%	89.4%	78.4%	97.8%
Diagnostic Accuracy	78.9%	86.5%	86%	71.7%	89.1%

Conclusion

Early diagnosis of bladder cancer is very important for improving outcomes, as bladder cancer ranges from noninvasive forms to invasive forms and can spread to other parts of the body. Urine cytology is a cheap, non-invasive OPD procedure with high specificity and low sensitivity. Because of its high specificity, we can advise urine cytology for each patient for early, accurate diagnosis and management.

***Abbreviations**:*

MGG – May-Grunwald Giemsa, PAP – Papanicolaou stain, NHGUC – Negative for High-Grade Urothelial Carcinoma, AUS – Atypical Urothelial Cell, LGUC – Low-grade urothelial neoplasm, HGUC – High-grade urothelial carcinoma, PUNLMP – Papillary urothelial neoplasm of low malignant potential

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Competing Interests: Nil

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