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ORIGINAL ARTICLE

Role of Urine Cytology in Accurate Diagnosis of Urothelial Carcinoma: An Immunohistochemical Study

Yousef A. Baumy [1], Masoud M. Omar [1], Raafat A. Hegazy [1], Naglaa A. Mostafa [1], Hamdy M. Desoky [2]

[1]Department of Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

[2]Department of Urology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

*corresponding author
Yousef Abd El Naby Baumy
pathology department, faculty of medicine, Zagazig University, Egypt.
Email: dr.yousefnousser@gmail.com
Phone number: 01061823793

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ABSTRACT

Background: The gold standards for detection of urothelial carcinoma are cystoscopy and biopsy. Both are invasive and expensive, therefore cytology is the first approach to investigate urothelial neoplasm, being safe and cost-effective.

Aim of the study: To evaluate the role of Minichromosome maintenance protein 2 in differentiation between benign and malignant urothelial lesions and the role of Fascin in detection of invasion in malignant tumors using urine cytology

Materials and Methods: A prospective study has been held from January 2016 to December 2018. Eighty cases divided into: group A included a definitive cytological diagnosis either benign or malignant and group B included a cytological diagnosis of atypia and suspicious. An immune-cytochemistry technique was done using minichromosome maintenance protein-2 and Fascin on urine cytology specimens for diagnosing bladder cancer.

Results: In group A the sensitivity of Minichromosome maintenance protein 2 and cytology were 90% and100% respectively, while the specificity was 100% and 70% respectively. In group B the sensitivity of MCM2 and cytology were 81% and 100% respectively, while specificity was 94.4% and 0.0% respectively. In both groups the sensitivity and specificity of Fascin were 86.5% and100% respectively.

Conclusions: Minichromosome maintenance protein 2 differentiates between benign and malignant tumors of the urinary bladder, also Fascin detect invasion by examination of urine cytology.

Keywords

Bladder cancer; Minichromosome maintenance protein 2; Fascin; urine cytology

INTRODUCTION

Urothelial carcinoma is the ninth most common disease globally, with 430.000 new cases and 165.100 deaths assessed in 2012; most of the bladder malignancy occurs in men[1]. Urothelial carcinoma is the dominating histologic type in the United States and Western Europe, where it represents roughly 90 percent of cancer bladder [2].

In Egypt, bladder cancer is the second malignancy in males, representing 12.7% among males' malignancy and 3.3% among females, as reported in the measurable yearly report of National Population-Based Cancer Registry Program [3]. High-grade urothelial carcinomas are regularly multifocal with a high mortality rate with muscle invasion. Consistent observation for urinary carcinoma is fundamental once the diagnosis is made [4].

Urine cytology acts as a complementary test to cystoscopy and biopsy. It is considered as a good test in recognizing high-grade urothelial carcinoma in the primary diagnosis. Urine cytology is the first specimen reviewed because of the high cost of both cystoscopy and biopsy [5].

Minichromosome maintenance protein 2 (MCM-2) is one of six related proteins that is important for DNA replication in eukaryotic cells. It additionally assumes a significant role

in genome stability during the S-stage. In the bladder, surface epithelial cells are exfoliated into the urine. Immuno-cytochemical staining for MCM-2 is used for discrimination between immuno-positive malignant cells and their immuno-negative normal counterparts [6].

The use of MCMs to distinguish bladder malignancy depends on the way that most cells in the human body, including urothelial cells, are not cycling and exist in a condition of quiet (G0 stage). Immuno-cytochemical staining for MCM-2 is utilized for clarifies its role [7].

Fascin is a 55-kDa globular protein that belongs to a unique family of actin binding proteins. Fascin contributes to the formation of various actins based structures, including the cellular surface protrusions that mediate cell movement. In vitro studies, elevated levels of Fascin were associated with increased speed of cell migration and emphasized the relationship between Fascin overexpression and motility of transformed cells [8].

Fascin expression is increased in human carcinomas compared with non-neoplastic epithelial lesions. Jin et al [9], have reported that Fascin overexpression may correlate with invasion and metastasis.

MATERIALS AND METHOD

Materials included in the study

Eighty patients with urothelial lesions were conducted at Departments of Pathology and urology, Faculty of Medicine, Zagazig University in the period from January 2016 to December 2018. All cases were selected from the urology department. The cases were identified based on clinical features Hematuria, frequency, dysuria, or history of bladder tumor. Types of specimens used were bladder wash. The study included urine samples and biopsy of patients with urothelial lesions. All samples are prepared as a cell block. The specimen is received fresh then just two drops are taken on 2-4 clean slides, spread of the smear is done by the blunt end of a syringe cover. The smear on a slide is left in air to a degree of about drying then fixed in 95% ethanol for minimum 20 minutes. The residual of specimen is left in a disposable centrifuge 5 ml tube for 15 minutes for clotting, then plasma or fluid is decanted gently. 10 % neutral buffered formalin is added to the tube containing clotted specimen at a ratio of (3/1), placed for 2 hours. The pellets of cells are produced by centrifugation at 3000 r.p.m for 10 minutes, and then cell blocks are prepared according to Hegazy methods [10].

Immunohistochemistry of MCM2 and Fascin

Serial sections from the cell blocks were submitted for immunohistochemical staining with MCM2 and Fascin. Paraffin sections were cut at 3-4 µ thickness and mounted on positively charged glass slides coated with N-poly L-lysine (1%). Paraffin sections were de-paraffinized by incubating them in the oven at 56 C for 15 minutes then inserted in xylene for 30 minutes. Rehydration of the slides in descending grades of alcohol: alcohol 95% for 5 minutes, alcohol 85% for 5 minutes and alcohol 75% for 5 minutes. Slides were rinsed in distilled water for 5 minutes. Washing sections in PBS for 5 minutes. Antigen Retrieval: sections were immersed in ready to use Dako target retrieval solution (PH 6.0) and at 80-90 c in a microwave for 5-10 minutes. Tapping off excess liquid using lint less tissue (such as gauze pad), carefully wipe around the specimen to remove any remaining liquid and to keep reagents within the prescribed area. Application of 3% Hydrogen peroxide to cover tissue sections and incubation for 5 minutes to block endogenous peroxidase. Sections were rinsed gently with distilled water. Tapping off excess liquid and wiping the slides as before. 2-3 drops of primary antibody (MCM2, Fascin) were placed on each slide and completely cover the specimen. The slides were incubated in humidity chamber overnight at 2-8 C. The slides were gently rinsed with buffer solution with avoiding direct flow on tissue. Tapping off excess buffer immediately and wipe slides as before. Sections were then incubated with biotinylated-antimouse immunoglobulin for 15 minutes at room temperature followed by washing in buffer. Sections were incubated with streptavidin-HRP for 15 minutes followed by washing. Diamminobenzidin substrate was

added to tissue sections; incubated for 5-10 minutes, then rinsed gently with distilled water. Slides were immersed in a bath of Mayer's hematoxylin (M.H); incubated for (2-5) minutes, depending on the strength of the used hematoxylin. Slides were gently rinsed in a distilled water bath. Slides were dipped 10 times into a bath of ammonia water to remove excess used hematoxylin stain. Slides were rinsed in a bath of distilled water for (2-5) minutes. Slides were cleared in xylene for 3 changes and finely mount with a cover slip using DPX.

Interpretation and evaluation of MCM-2 immunostaining

Nuclear positivity of medium and high intensity (2+ and 3+ on 0 to 3 scales) is considered as positive. No intensity (0) and mild intensity (1+) are considered as negative. Absolute numbers of positive cells within ten consecutive high-power fields (at magnification 400x) were counted in all controls [11]**.**

Interpretation and evaluation of Fascin immune-staining

Cytoplasmic positivity of score 0: Absent, score 1: 25%, score 2: 25%-50%, score 3: 50%-75% and score 4: >75%. No intensity (0), weak intensity (1+), moderate (2), strong (3) [12]**.**

The immune-reactivity score was calculated by multiplying the score for extent by the score for intensity for each case. Grouped as: Absent (0): 0, Mild staining (1): 1-4, Moderate (2): 5-8 and Intense (3): 9-12

Ethical Considerations

A written consent was obtained from all cases. This work has been carried out following the Code of Ethics of the World Medical Association (Helsinki Declaration of 1975, as revised in 2000) for humans' studies. Institutional Review Board (IRB) of the faculty of Medicine Zagazig University affirmed the study protocol (No. 2363)[13].

Statistical analysis

Data on laboratory investigations and outcome measures were collected and coded using Microsoft Excel sheet. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 20.0 for windows. P-value <0.05 indicates significant results and <0.001 is considered highly significant results.

RESULTS

The cases in group A were in the age group of 38 to 80 years, with mean age, was 64.4 years, Expression of MCM2 in benign and malignant urothelial lesions diagnosed by histopathology (group A) in comparison to cytology **shown in Table 1**

The sensitivity of MCM2 and cytology were 90% and 100% respectively while the specificity were 100% and 70% respectively,

The cases in group B were in the age group from 44 to 85 years; with mean age was 64.3 years. Expression of MCM2 in benign and malignant urothelial lesions diagnosed by histopathology (group B) in comparison to cytology (**shown in Table 2**)

The sensitivity of MCM2 and cytology were 81% and 100% respectively while the specificity were 94.4% and 0.0% respectively,

Immmunohistochemical expression of Fascin in invasive and non-invasive malignant lesions of group A and B **shown in Table 3 and 4**

The sensitivity and specificity of Fascin in both groups were 86.5% and 100% respectively.

Immunohistochemical expression of MCM2 and Fascin in urothelial carcinoma

Figure 1: MCM2 nuclear immune-staining of urothelial carcinoma score 3 400X

Figure 2: MCM2 nuclear immune-staining of urothelial carcinoma score 2 400X

Figure 3: Fascin cytoplasmic immune-staining of urothelial carcinoma score 3 400X

Table (1): Expression of MCM2 in benign and malignant urothelial lesions diagnosed by histopathology (group A) in comparison to cytology

Variables	Total (n=20) Cytology	Histopathology		Test of sig.	P
		Benign (n=10)	Malignant lesions (n=10)		
Cytology:				X^2	**0.001**
Negative	7(35.0%)	7(100.0%)	0 (0.0%)	12.5	**HS**
Low-grade cancer	5 (25.0%)	3 (60.0%)	2 (40.0%)		
High-grade cancer	8 (40.0%)	0 (0.0%)	8 (100.0%)		
MCM2:				X^2	**<0.001**
Positive	9 (45.0%)	0 (0.0%)	9 (45.0%)	fisher	**HS**
Negative	11 (55.0%)	10 (50.0%)	1 (5%)		

Table (2): Expression of MCM2 in benign and malignant lesions in comparison to cytology in group B

Variables	Total (n=60)	Histopathology		Test of sig.	P
		Benign (n=18)	Malignant (n=42)		
Cytology:				X^2	**<0.001**
Suspicious	22 (36.7%)	0 (0.0%)	22 (100%)	fisher	**HS**
Atypia	38 (63.35)	18 (47.3%)	20 (52.6%)		
MCM2:				X^2	**<0.001**
Positive	35 (58.4%)	1 (1.6%)	34 (56.6%)	fisher	**HS**
Negative	25 (41.6%)	17 (28.3%)	8 (13.3%)		

Table (3): Immmunohistochemical expression of Fascin in invasive and non invasive malignant lesions of group A

Fascin	Total (n=10)	Histopathology		X^2	P
		Invasive lesions (n=8)	Noninvasive lesions (n=2)		
Positive	7 (70.0%)	7 (70.0%)	0 (0.0%)	fisher	0.06
Negative	3 (30.0%)	1 (10%)	2 (20%)		

Table (4): Immmunohistochemical expression of Fascin in invasive and non invasive malignant lesions of group B

Fascin	Total (n=42) Cytology	Histopathology		X^2	P
		Invasive lesions (n=29)	Noninvasive lesions (n=13)		
Positive	25 (59.5%)	25 (59.5%)	0 (0.0%)	fisher	**<0.001 HS**
Negative	17 (40.5%)	4 (9.5%)	13 (30.9%)		

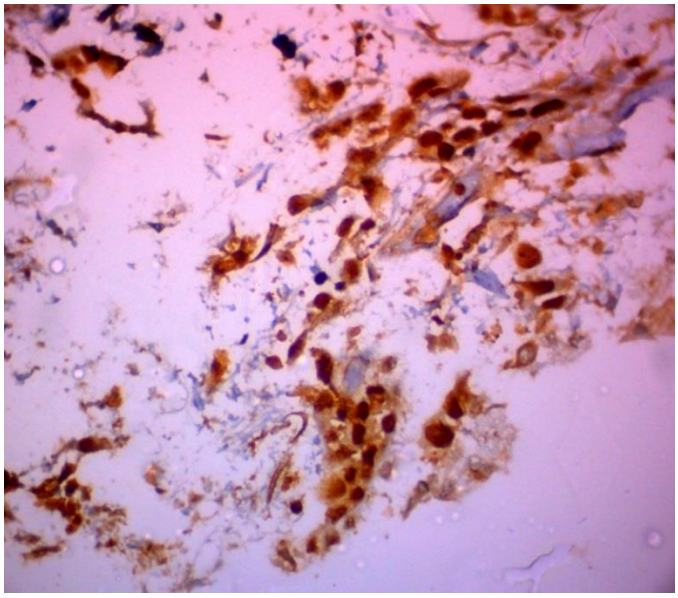

Figure 1 High grade urothelial carcinoma: MCM2 immune staining shows positive nuclear reaction with high intensity of the cell clusters scor 3 (*Mayer's hematoxylin background. DAB chromogen x 400*

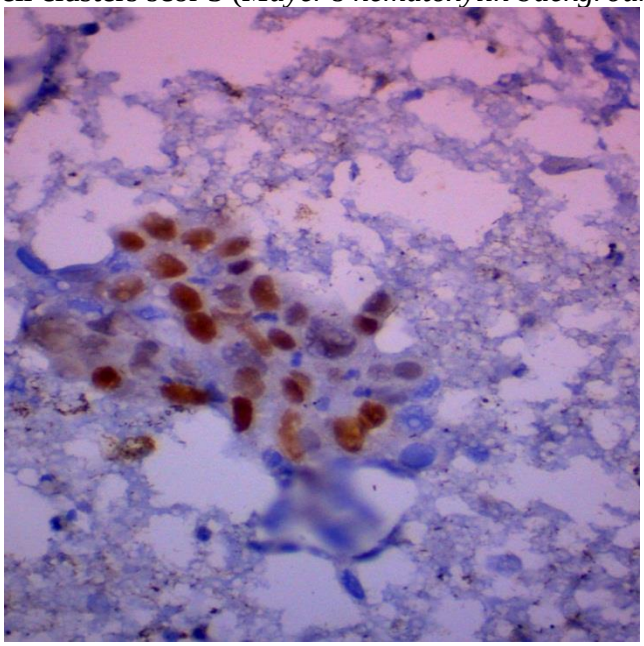

Figure 2 High grade urothelial carcinoma: MCM2 immune staining shows positive nuclear reaction with moderate intensity of the cell clusters scor 2 (*Mayer's hematoxylin background. DAB chromogen x 400*

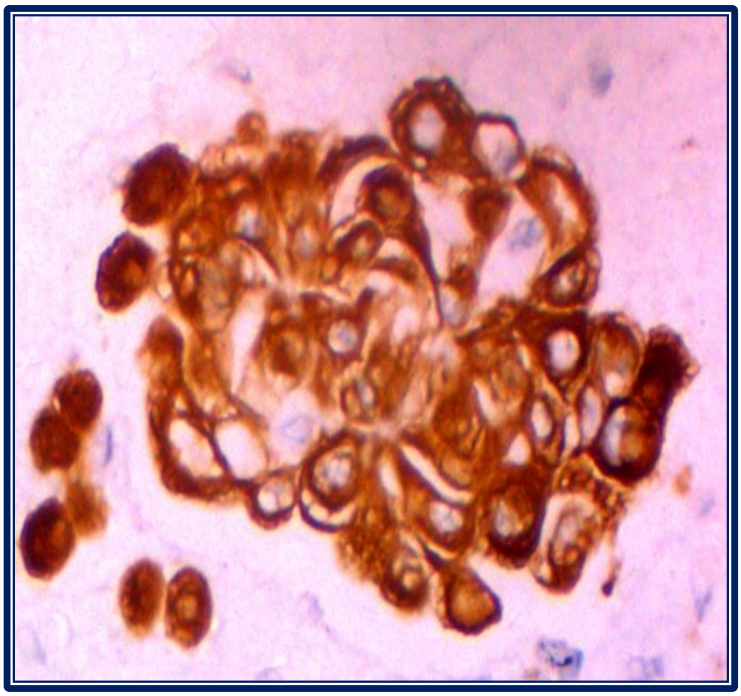

Figure 3 High grade urothelial carcinoma: Fascin immune staining shows positive cytoplasmic reaction with strong intensity of the cell clusters scor 12 (*Mayer's hematoxylin background. DAB chromogen x 400)*

DISCUSSION

Urine cytology had an important role in the detection of urothelial carcinoma in high-risk patients [14]**.** When a diagnosis of atypia or suspicious is found, invasive procedures may lead to patient's discomfort and high cost. A cell block is considered a useful adjunct for the diagnosis of low-grade urothelial neoplasia (LGUN) and negative for high-grade urothelial carcinoma [15].

In this study, we evaluated immune-cytochemical staining of urothelial cells with MCM2 and Fascin. These markers have been used as molecular markers for differentiating neoplastic/dysplastic cells in the gynecological lesions. We demonstrate that these markers could also serve as adjunct markers in urine cytology. MCM2 and Fascin staining results showed high correlation between the cytology and the histopathological diagnosis by which definitive diagnoses have been made in group A. so performing cytological diagnosis using these markers could replace the invasive method of obtaining biopsies.

the sensitivity, specificity , accuracy, positive predictive value and negative predictive value of cytology was as follow, 100%, 70.0%, 85.0%, 76.9% and 100% respectively in group A

the sensitivity, specificity, accuracy, positive predictive value and negative predictive value of cytology in group B was as follow, 100%, 0.0%, 70.0%, 100% and 0.0% respectively.

The sensitivity, specificity, accuracy, positive predictive value and negative predictive value of cytology in both group 100%, 25 %, 73.8%, 71.2%, and 100% respectively. Near to our results Green et al [16]. Stated that, cytology alone by cell block has low specificity 35% while sensitivity was 92.1% in detecting urothelial carcinoma.

Against our study Garbar et al [17] who found that between 2002 and 2004, 592 bladder washings were obtained using a flexible cystoscope. The overall sensitivity 37% % and specificity 99%. This may be due to usage of large number of cases in this study.

This short review focus on the employment of MCM2 to improve detection of urothelial carcinoma in urine cytology specimen. MCM2 is over-expressed in the cell nucleus during

aberrant S-phase induction of human papillomavirus HPV)–infected cells [19].

MCM2 protein is a key component in the formation of replication forks and recruitment of other DNA replication related protein, deregulation of MCM2 function has been suggested to contribute to tumorgenesis [18].

The sensitivity, specificity, accuracy, positive predictive value and negative predictive value of MCM2 in group A were as follow, 90%, 100%, 95.0%, 100% and 90.9% respectively.

While in group B it was 81%, 94.4%, 85.0%, 97.1% and 68.0% respectively. And in both groups they 82.7%, 96.4%, 87.5%, 97.7% and 75.0% respectively. Near to our results, Saeb-Parsy et al [20] stated that those values of MCM2 in urine cytology specimens were 95%, 95%, 92.7% and 89.7 respectively.

Fascin, an actin binding protein induces membrane protrusions, enhances epithelial cell motility, and is highly expressed in transformed epithelial cells as well as in specialized normal cells [21].

Fascin expression in normal urothelium was absent however showed intense immune-reactivity in neoplastic urothelium, both in invasive and non-invasive areas. Fascin plays an important role in migration and invasion of the urothelial carcinoma. Down regulation of fascin reduces cell migration and invasion [12]. Our study shows that targeting Fascin may be a novel therapeutic strategy for urothelial carcinoma of the bladder

The sensitivity, specificity, accuracy, positive predictive value and negative predictive value of Fascin in both groups was 86.5%, 100%, 90.4%, 100% and 75.0% respectively. Agreeing with our results, McKnight et al [22], who stated that t the sensitivity, specificity, of Fascin in urine cytology were 100%. Foteini et al [23], found that Fascin expression was significantly higher in invasive UCs. In addition, they observed a strong staining exclusively in invasive carcinomas and that none of the pTa tumors demonstrated intense staining

In our study, the sensitivity of cytology and MCM2 were 100% and 90% in group A respectively, while the specificity of cytology and MCM2 were 70% and 100% in group A, respectively. Moreover, the sensitivity of cytology and MCM2 were 100% and 81% in group B respectively, while the specificity of cytology and MCM2 were 0.0% and 94.4% in group B. Thus, we suggest that MCM2 has a minor role in group A while MCM2 had an essential role in group B when the cytological diagnosis was atypia or suspicious.

CONCLUSIONS

Urine cytology by cell block is simple and non-invasive diagnostic test used for detection of the early cases of bladder cancers. Cell blocks resolve the problem of cytology in group A, as the sensitivity and specificity was 100% and 70%, but its defect in group B when the diagnosis was atypia or suspicious the specificity was 0.0 % in group B and 25% in all cases. MCM2 resolve this problem, where the sensitivity and specificity were 81% and 94.4% respectively in group B, in all cases the sensitivity and specificity were 82.7% and 96.4% respectively. The confidence is around 80 %. Fascin confirm the results of the marker by its positivity in urothelial groups with invasion and differentiate invasive groups from non-invasive by urine cytology without surgical intervention.

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