Egypt. J. Chem. **Vol. 67**, No. 7 pp. 581 - 588 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Evaluation of Minichromosome Maintenance Deficient-5 Levels in non-Muscle invasive Bladder Cancer Patients

Faten Z Mohamed Ibrahim[a], Reda M Abdelal[b], Ehab M Elbadry[c]*, Waleed M Serag[b]

[a]*Biochemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt*
[b]*Chemistry Department, Faculty of Science, Suez University, Suez, Egypt*
[c]*Clinical Pathology Department, Damietta Cancer Institute, Damietta, Egypt*

Abstract

Objectives: The diagnosis of bladder carcinoma is currently made using cytology and cystoscopy, which pose a significant challenge for clinicians due to a lack of sensitivity and specificity; therefore, we examine how well Minichromosome Maintenance Deficient-5 factor (MCM-5) in various body fluids (serum, urine, and tissue homogenate) can detect non muscle invasive bladder cancer (NMIBC) patients.

Patients: At Damietta Cancer Institute, Damietta, Egypt, Fifty patients and thirty healthy subjects were recruited and patients pathologically diagnosed as non-muscle invasive bladder cancer. All subjects had their serum, urine, and tissue homogenate marker levels determined.

Results: The levels of Minichromosome Maintenance Deficient-5 factor were significantly higher in urine and tissue homogenate samples from Ta and T1 non muscle invasive bladder cancer (NMIBC) patients compared to the control group ($p< 0.001$), and serum MCM5 levels were significantly higher compared to the control group ($P =0.036$). Urine MCM-5 had a higher negative predictive value (NPV) and positive predictive value (PPV) than the other fluids (serum and tissue homogenate) and could be used as a predictive marker for the recurrence of NMIBC.

Conclusions: The urinary Minichromosome Maintenance Deficient-5 assay is a simple and inexpensive test that may be useful as a biomarker for the diagnosis of non-muscle invasive bladder cancer patients and to determine patients who need to have their cystoscopy repeated.

Keywords: Bladder cancer; NMIBC; MCM5; NPV; PPV.

1. Introduction

Cancer is the world's second leading cause of mortality in the 21st century. In comparison to other diseases, cancer kills approximately one out of every six deaths worldwide, according to the American Cancer Society Cancer Facts [1-4]. Cancer is one of the most serious human health problems and considered the second major reason for death all over the world. Cancer is widely progressed in the modern era and is expected to hit ~25 million people in the next 20 years [5-8].

Bladder cancer is considered the sixth most

*Corresponding author e-mail: ehab.m.elbadry@gmail.com.; (Ehab Mohamed Elbadry).
EJCHEM use only: Received date 11 November 2023; revised date 19 December 2023; accepted date 15 January 2024
DOI: 10.21608/EJCHEM.2024.247314.8834

prevalent tumor in males, the seventeenth most prevalent cancer in women, and the tenth most prevalent tumor in both sexes worldwide, with a displayed 573,278 new cases and 212,536 deaths in 2020[9]. 75% of the newly diagnosed bladder cancer is non-muscle invasive bladder cancer (NMIBC). These tumors are either sub mucosal (T1 stage) or bladder mucosal (Ta stage and carcinoma in situ CIS) [10]. NMIBC had a greater expected survival than muscle invasive bladder cancer (MIBC) (T2-T4 stages), even after undergoing a radical cystectomy as treatment [11].

NMIBC, on the opposite hand, has a high overall recurrence rate. Necessitating regular endoscopic controls that are both painful and costly. Cystoscopy is a problematic technique that can result in painful urination (50%), increased urination (37%), and hematuria (19%) [12]. Furthermore, the sensitivity of urine cytology is low, particularly with low grade tumors [13].

Cytology has a broad variability among observers and can be difficult to differentiate between atypical and inflammatory or infectious alterations [14]. The high impact recurrence rate of NMIBC is often described by the European Organization for Research and Treatment of Cancer (EORTC) risk score, which varies from 31 to 78% [15]. It requires a precise surveillance program for early detection and treatment [16].

Urine biomarkers with lower sensitivity and specificity have been approved by the Food and Drug Administration (FDA) [17], which represents an obstacle for their routine clinical acceptance. The field of genetic urine biomarker research is growing. The biomarker, which is not the true clinical gold standard, is compared with voided cytology in the majority of published research. Furthermore, as biomarkers' performance cannot enhance cystoscopy and cytology performance, the 2022 EAU Guidelines do not advise their use in a monitoring program for high-risk NMIBC[17,18].In the groups at intermediate and low risk The Food and Drug Administration (FDA) approved urine biomarkers that have lower sensitivity and specificity [17], making their usage a challenge in daily clinical practice.

EAU Guidelines published in 2022 state that a new surveillance strategy for high-risk NMIBC is not indicated to use biomarkers because their performance cannot enhance cystoscopy and cytology performance. They believe that several of the recently developed biomarkers may be used to substitute or delay cystoscopies in the intermediate and low-risk populations, despite the lack of high-quality evidence. Recurrences in these groups are often low-grade, and biomarkers are able to detect an unusually high-grade recurrence in this case with high negative predictive value and sensitivity [18].

There is an effort towards developing new urine biomarkers because cystoscopy and cytology are the only clinical options available for use as surveillance techniques for high-risk NMIBC. In fact, a few of these urine markers are already FDA-approved, but regrettably, none of them have managed to make it into the clinical practice guidelines [19]. The specificity and positive predictive value of new modern biomarkers are often poor, but their negative predictive value and sensitivity for high-grade recurrences are over 90% [18, 20, and 21]. To achieve this, it would be necessary to develop a test with a high NPV to make sure that tumors with a higher risk of growth are not missed [17]. Although several urine biomarkers have been created with this objective in mind, none have been used in clinical practice because of poor performance and a lack of high-quality prospective validation studies [22].

Minichromosome maintenance (Mcm) family proteins (Mcm2-7), collectively known as MCM, form hexameric complexes with DNA helicase activity, which is essential for DNA synthesis to begin [23]. MCM proteins are overexpressed and deregulated in malignant states epithelial-lined organ systems [24, 25].

DNA licensing factor MCM5, a proliferative biomarker, has previously been proven to be a highly accurate biomarker in the detection of bladder cancer [26]. All cells with the ability to proliferate express MCM5, but in a healthy urothelium, MCM5 expression is only found in cells that line a typical bladder and release into urine are MCM5 negative. In urothelial carcinomas, where cells proliferate out of control and are exfoliated from the bladder surface, MCM5 expression is present at all levels of the urothelium in urothelial carcinomas, where cells proliferate excessively, resulting in cells that express MCM5 being exfoliated from the bladder surface.

A tumor appears when MCM5-positive cells are seen in urine sediment. In fact, it has been shown that in individuals with bladder cancer, the degree of Mcm5 expression can predict both death and

recurrence [27].

MCM5 detection in urine sediment has already been shown to be a promising marker for bladder cancer, with high sensitivity and negative predictive value [26, 28]. Instead of employing MCM5-positive cells in urine, numerous commercially available MCM5-enzyme-linked immunosorbent tests may detect MCM5 in urine sediment in bladder cancer patients, paving the path for further study into MCM5 as a new biomarker.In fact this the first study contributes MCM5 levels in tissue homogenate samples.

2. Subjects and Methods

2.1. Subjects

Patients in this study were first diagnosed pathologically at the Damietta Cancer Institute's Pathology Department in Damietta, Egypt. Bladder cancer can be divided into NMIBC and MIBC, non-muscle invasive bladder cancer (NMIBC), which is limited to the mucosa layer (Ta) or sub mucosa (T1), or CIS. MIBC is defined as T2, T3, and T4 as shown in (Fig.1).

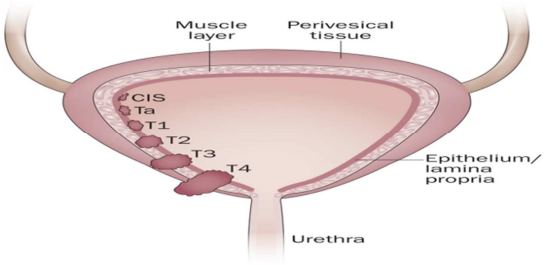

Figure 1: Bladder cancer staging [29]

Eighty subjects were selected and divided into three groups: Ta NMIBC (n = 26), T1 NMIBC (n = 24), and thirty healthy subjects (Table 1).

All subjects had their serum, urine, and tissue homogenate samples taken.

All procedures involving human subjects in the study were carried out in compliance with the ethical standards of the Suez University Ethics Committee, Suez, Egypt (IRB no. 151222). (Table 1) summarized the characteristics of the patients and control groups, which were as follows: 39 male and 11 female cases; 25 cases diagnosed as Ta NMIBC (papillary non-invasive tumor) patients; 25 cases diagnosed as T1 NMIBC patients; and the normal control group included 30 cases that were age and gender matched. CIS NMIBC not included in this study due to the low sample size.

Table 1
Characteristic features of patients and control groups

	Control	Ta NMIBC	T1 NMIBC
Age: Mean±SD (range)	56 ±11 (43-66)	60 ±8 (50-66)	62±9 (51-69)
Number of cases	30	25	25
Male: Female ratio	21:9	22:3	17:8

Patients had to be between the ages of 43 and 69 and have never previously received radiation or chemotherapy. Patients, who present with other urinary system malignancies, severe diseases that are obvious in other systems, or autoimmune diseases, including systemic lupus erythematous, rheumatoid arthritis, and others, are prohibited from the study. Patient's clinical data, including age, gender, tumour pathology, and stage, were obtained from medical records. To play out the pathological staging of the study, the TNM (Tumour, Nodes, and Metastasis) classification system for bladder cancer was used [30]. All samples had their serum, urine, and tissue homogenate MCM5 levels measured using an competitive enzyme-linked immunosorbent assay (ELISA), Statistical analyses were used to uncover the relationships.

2.2. Samples Preparation:

Venous blood (5–10 mL) and fresh mid-stream urine (10–20 mL) were collected in the morning. All samples were centrifuged for 15 minutes at 3000 rpm, and the supernatants were immediately stored at 80 °C.

Tissue samples were collected and weighted after cutting, then PBS was added (PH7.2–7.4), rapidly frozen with liquid nitrogen, maintained at 2–8 °C after melting, added PBS (PH7.4), homogenised by hand or grinders, and centrifuged for 20 minutes at a speed of 2000–3000 r.p.m. to remove supernatant.

The enzyme-linked immunosorbent assay (ELISA) is used to measure the levels of MCM5 in serum, urine, and tissue homogenate (SHANGHAI KORAIN BIOTECH CO., LTD, SHANGHAI, CHINA).Cat No E4853Hu.

Statistical analysis

T-tests and ANOVA tests were used to compare continuous variables with normally distributed patterns between groups. ROC analysis was utilised to identify the diagnostic values of the markers. The statistical programme SPSS v.15.0 (SPSS Inc., USA) was used to analyse all of the data. Alpha values of

P<0.05 were considered statistically significant [31].

3. Results

MCM5 levels were significantly elevated in Ta NMIBC patients urine and tissue homogenates (266.4±95.1 pg/ml and 66.3±22.6 pg/g.tissue, p < 0.001), respectively, compared to the control groups (25.2±10.4 pg/ml and 7.78±2.6 pg/g.tissue), respectively, as well as in serum samples (3.8±0.9 pg/ml) compared to its levels in the control group (3.1±1.1 pg/ml, P value was 0.036) Table 2, Figure 2.

Table2

MCM5 concentration in control group, Ta Non muscle invasive bladder cancer, T1 Non muscle invasive bladder cancer patients group

Marker	Control	Ta	T1
MCM5(Urine) pg/ml P value	25.2±10.4	266.4±95 P< 0.001	302±86 P< 0.001
MCM5(Serum) pg/ml P value	3.1±1.1	3.8±0.9 P=0.036	3.8±1.1 P=0.03
MCM5(Tissue homomgenate) pg/g.tissue P value	7.78±2.6	66.3±22.6 P<0.001	84.6±20.6 P< 0.001

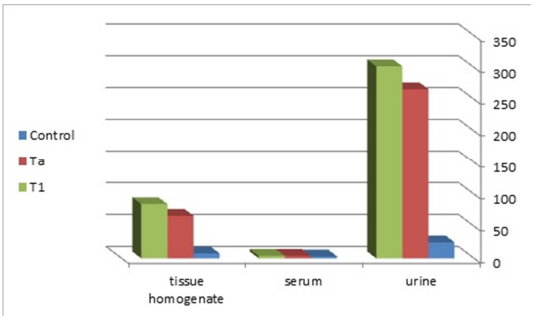

Figure 2: MCM5 concentration in control group, Ta Non muscle invasive bladder cancer, T1 Non muscle invasive bladder cancer patients group

Furthermore, our findings revealed a significant elevation of the MCM5 levels in the urine and tissue homogenate of T1NMIBC patients (302±86.2 pg/ml, 84.6±20.6 pg/g. tissue, p < 0.001), respectively, compared to the control group (25.2±10.4 pg/ml, 7.78±2.6±2.1 pg/g.tissue), but the significance was less in the serum samples (3.8±1.1 pg/ml, P value of 0.03) compared to the control group (3.1±1.1 pg/ml). (Table 2, Figure 2).

Our findings revealed that there was a strong correlation between urine and serum MCM5 levels and NMIBC (r = 0.48**, P<0.001) (Table 3, figure 3); as well as between urine and tissue homogenate MCM5 levels (r = 0.74**, P<0.001) (Table 3, figure 4); and serum and tissue homogenate MCM5 levels (r = 0.26*, P<0.05) (Table 3, figure 5).

Table 3

Pearsons's correlation analysis between MCM5 in urine, serum and tissue homogenate of non muscle invasive bladder cancer Patients

	MCM5 Urine	MCM5 Serum	MCM5 Tissue homogenate
MCM5 Urine	-	0.48**	0.74**
MCM5 Serum	0.48**	-	0.26*
MCM5 Tissue homogenate	0.74**	0.26*	-

*Correlation is significant at the 0.05 level

**Correlation is highly significant at the 0.01 level

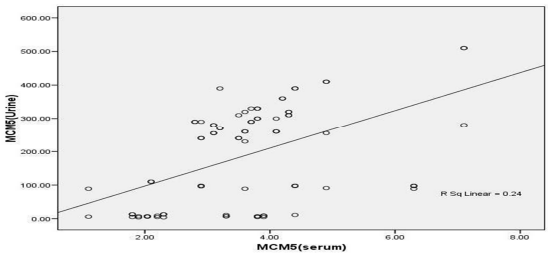

Figure 3: Correlation between urine MCM5 and serum MCM5 which indicated strong positive correlation (r= 0.48**) (P< 0. 001)

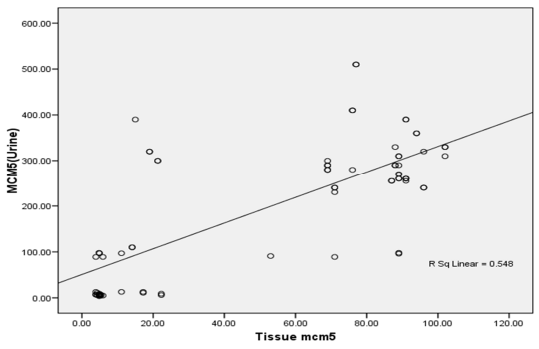

Figure 4: Correlation between urine MCM and tissue homogenate MCM5 which indicated strong positive Correlation (r= 0.74**) (P< 0.001)

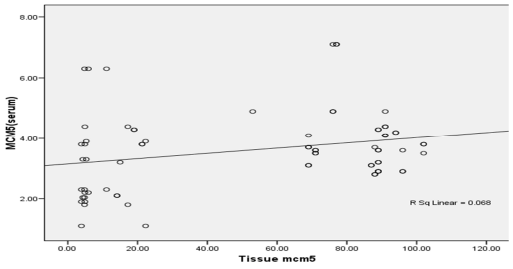

Figure 5: Correlation between tissue homogenate MCM5 and serum MCM5 which indicated a positive correlation (r= 0.26*) (P< 0.05)

According to ROC analysis, the AUC in MCM5 urine samples from Ta NMIBC patients was 0.98 (95% CI: 0.98–1.0), P<0.001(Figure 6), the AUC in Ta NMIBC patients' serum MCM5 samples was 0.67 (95% CI: 0.52-0.84), P = 0.038 (Figure 7), and the AUC in Ta NMIBC patients' tissue homogenate MCM5 samples was 0.96 (95% CI: 0.96–1.0) P< 0.001(Figure8). In the diagnosis of Ta NMIBC patients, urine, serum, and tissue homogenate sensitivities were 88.5%, 73%, and 92.3%, respectively, while specificities were 97%, 60%, and 83.3%, respectively. Table 4

Table 4
ROC curve evaluation between MCM5 in control group (serum, urine and tissue homogenate) and that of Ta NMIBC patients group

Marker	AUC	Std. Error	95 % CI	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
MCM5 Urine	0.98	0.006	0.98-1.0	90.5	97	98.2	95.9	94	P<0. 001
MCM5 Serum	0.67	0.08	0.52-0.84	73	60	61.2	72	66	0.038
MCM5 tissuehomogenate	0.96	0.007	0.96-1.0	92.3	83.3	85.7	90.1	88	P<0.001

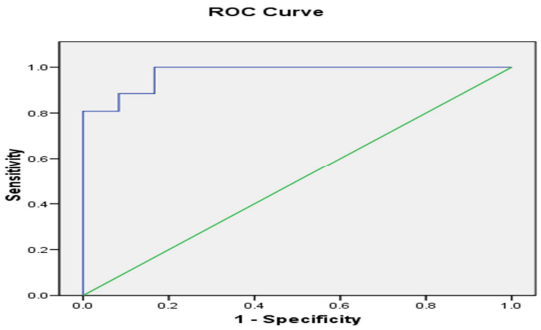

Figure 6: ROC curve for urinary MCM5 in control group versus Ta NMIBC patients group

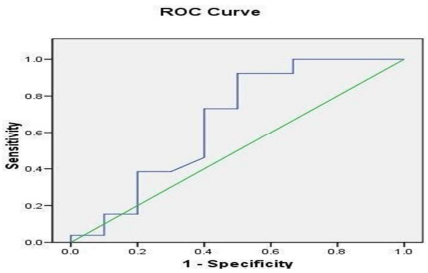

Figure 7: ROC curve for serum MCM5 in control group Versus Ta NMIBC patients group

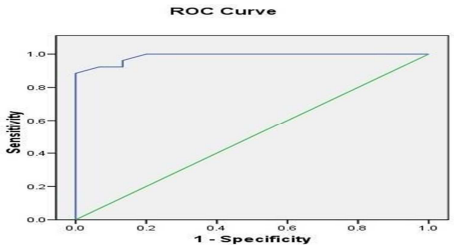

Figure 8: ROC curve for tissue homogenate in control group versus Ta NMIBC patients

ROC analysis revealed that the AUC in urine of T1 NMIBC patients was 0.97 (95% CI: 0.96–1.0) P<0.001(Figure 9), the AUC in serum was 0.68 (95% CI: 0.52-0.84) P=0.029 (Figure 10), and the AUC in tissue homogenate was 0.96 (95% CI: 0.96–1.0) P<0.001 (Figure 11). The sensitivities of urine, serum, and tissue homogenate in the diagnosis of T1 NMIBC patients were 95.8%, 62.5%, and 97%, respectively, with specificities of 98%, 60%, and 83.3%. Table 5

Table 5
ROC curve evaluation between MCM5 in control group (serum, urine and tissue homogenate) and that of T1 NMIBC patients group

Marker	AUC	Std. Error	% 95CI	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
MCM5 Urinee	0.97	0.006	0.96-1.0	95.8	98	98.2	96.7	98	P<0.001
MCM5 Serum	0.68	0.08	0.52-0.8	62.5	60	55.5	66.6	61	0.029
MCM5 tissuehomogenate	0.97	0.007	0.97-1.0	97	83.3	85.7	97.4	91	P<0. 001

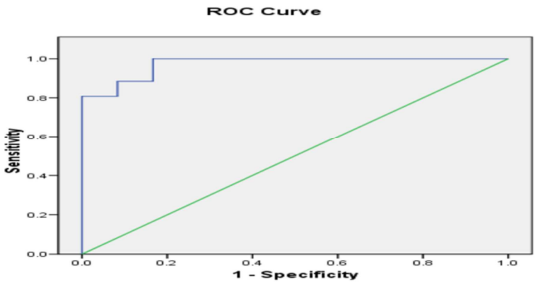

Figure 9: ROC curve for urinary MCM5 in control group versus T1 NMIBC patients group

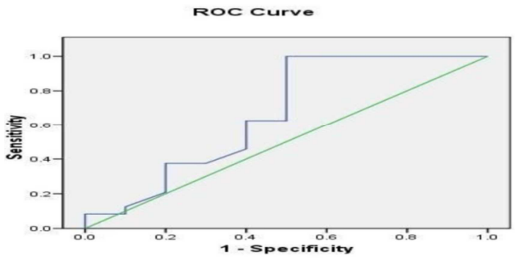

Figure 10: ROC curve for Serum MCM5 in Control group versus T1 NMIBC patients group

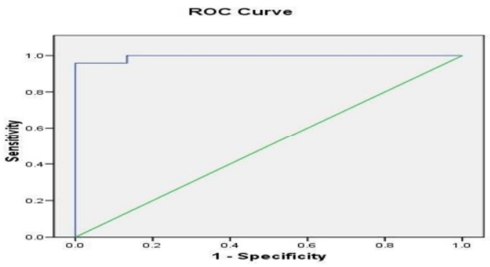

Figure 11: ROC curve for tissue homogenate MCM5 in control group versus T1 NMIBC patients

4. Discussion

Although there is growing evidence that new urine biomarkers perform well for NMIBC surveillance, none have been established as a viable replacement for the gold standard of cystoscopy and bladder cytology [18]. According to the EAU Guidelines of 2022, for the first time, it has been recognized that patients originally diagnosed with TaG1-2 bladder cancer may benefit from employing biomarkers or bladder ultrasounds for surveillance if cystoscopy is not feasible or the patient declines to have one [18]. However, urinary indicators cannot substitute cystoscopy during follow-up or lower the frequency of cystoscopies, according to EAU Guidelines from 2021[10].

MCM5 can be detected in urine if MCM5-containing cells are present. The difference in sensitivity of the MCM5 test in detecting recurrent tumors versus the diagnostic indication (45% recurrence Bladder cancer versus 73% primary Bladder cancer) could be explained by the fact that most recurrent tumors are smaller and lower grade, and smaller, lower-grade tumors shed fewer cells into the urine than larger, higher-grade tumors [32], which matched with our findings that levels of the marker increased with The sensitivity of the MCM5 test for recurrent Ta low-grade tumors was lower than that for primary tumors, despite this [28], and this is matched with our findings that levels of the marker increased with tumor stage.

In fact, the inhibition of MCM5 expression in bladder cancer and OSCC cells resulted in the downregulation of CDK and cyclin E expression and upregulation of p21 expression, which may led to G2/M phase arrest in bladder cancer and OSCC cells. These results further verified that MCM5 is highly expressed in patients with bladder cancer, which promotes the proliferation of bladder cancer cells and regulates cell cycle [33].

Although the MCM5 test's sensitivity for recurrent Ta low-grade tumors was lower than that for primary tumors [28], only 2% of low-risk NMIBC patients develop within 10 years, on average, due to the slow growth and low risk of progression of small, recurrent, low-grade tumors [34].

In fact, our findings showed that low-grade tumors (Ta NMIBC) have lower levels of MCM5 in urine, serum, and tissue homogenate when compared to T1NMIBC. This could be explained by the fact that low-grade tumors release fewer MCM5 containing cells into the urine.

Furthermore, according to EORTC (European Organization for Research and Treatment of Cancer), MCM5 detection has a far higher ability to identify intermediate and high risk patients, with a sensitivity of 75.6% and a negative predictive value of 99%.

Our results demonstrated that urinary MCM5 has a sensitivity of 90.5% and a NPV of 95.9% in Ta NMIBC and a sensitivity of 95.8% and a NPV of 96.7% in T1 NMIBC. This could imply that MCM5 is an important marker in the follow-up of NMIBC

patients, particularly T1 NMIBC, as it has a high NPV for both Ta and T1 NMIBC, 95.9% and 96.7%, respectively, and could reduce regular cystoscopies, as many urologists currently perform more regular cystoscopies than is recommended in guidelines, especially in low-risk disease, as a precautionary measure [28].

Except for a negative cystoscopy, there is presently no consensus on how to treat high-risk NMIBC with a positive urine test. Given the possibility of progression in these individuals, we suggest that high-risk patients with an abnormal MCM5 test result have another cystoscopy performed in accordance with the guidelines. The follow-up regimen and surveillance rhythm could be altered with a negative MCM5 result, preventing needless cystoscopies for individuals at low risk of progression, with a negative MCM5 result ruling out a high-risk tumor.

Similarly, MCM5 detection appears to be anaccurate, simple, and noninvasive test for identifying patients with pancreatic biliary and prostate cancer malignancies [26]. It was reported that that p53 negatively regulates MCM5 expression and its transcriptional regulator E2F1 and plays a role in negating p53's growth arrest function [28]. Indeed, it was reported that the estrogen receptor modulated MCM5 to promote bladder cancer development in vitro and in vivo [31], which could explain MCM5's role in tumorigenesis. Indeed, our findings show that urinary MCM5 has the best diagnostic power for detecting Ta and T1 NMIBC patients, with AUCs of 0.98 and 0.97, respectively. Tissue homogenate samples may also be useful in diagnosis.

5. Conclusions

The urinary MCM5 assay is a simple and inexpensive test that may be useful as a marker for the diagnosis and exclusion of NMIBC patients who need to have their cystoscopy repeated with NPV of 95%.

6. Conflict

Authors declare that they have no conflict of interest.

7. References

[1] Mahdy A, Ali O, Serag W, Fayad E, Elshaarawy R, Gad E. Synthesis, characterization, and biological activity of Co (II) and Zn (II) complexes of imidazoles-based azo-functionalized Schiff bases. Journal of Molecular Structure. 2022,1259:132726.

[2] Serag W, Zahran F, Abdelghany Y, Elshaarawy R, Abdelhamid M. Synthesis and molecular docking of hybrids ionic azole Schiff bases as novel CDK1 inhibitors and anti-breast cancer agents: In vitro and in vivo study. Journal of Molecular Structure. 2021;1245:131041.

[3] Mohamed B, Serag W, Abdelal R, Elsergany H. S100A14 protein as diagnostic and prognostic marker in hepatocellular carcinoma. Egyptian Liver Journal. 2019;9(1):1-6.

[4] Serag W, Elsayed B. Annexin A5 as a marker for hepatocellular carcinoma in cirrhotic hepatitis C virus patients. Egyptian Liver Journal. 2021;11(1):32.

[5] Serag W. The relation between Helicobacter pylori infection and hepatocellular carcinoma in Egyptian patients. American Journal of Biochemistry & Biotechnology. 2015;11(2):110.

[6] Serag W, Elsayed E. Cardiac Status in Hepatitis B Virus and Hepatocellular Carcinoma in Egyptian Adolescents. Biochemistry Letters. 2017;13(1):198-208.

[7] Serag W, El Sayed B. The Clinical Impact of Serum Testosterone Levels in Egyptian Patients with Different Stages of Hepatitis C Virus. Serag WM, El Sayed BE. The Clinical Impact of Serum Testosterone Levels in Egyptian Patients with Different Stages of Hepatitis C Virus. Egy. J. Pure & Appl. Sci. 2017; 55(3):39-44

[8] Serag W. Elevated Alpha Fetoprotein in Chronic HCV Liver Disease with and without Hepatocellular Carcinoma in Egyptian Patients. Journal of Dental and Medical Sciences (IOSRJDMS) e-ISSN. 2014:2279-0853.

[9] Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 2021, 71, 209–249.

[10] Compérat E, Burger M, Gontero P, Mostafid AH, Palou J, Rouprêt M, et al. Grading of Urothelial Carcinoma and The New "World Health Organisation Classification of Tumours of the Urinary System and Male Genital Organs 2016". Eur Urol Focus. 2019; 5 (3):457-466.

[11] Chen J, Zhang H, Sun G, Zhang X, Zhao J, Liu J, Shen P, Shi M, Zeng H. Comparison of the prognosis of primary and progressive muscle-invasive bladder cancer after radical cystectomy: A systematic review and meta-analysis. Int. J. Surg. 2018; 52, 214–220.

[12] Burke D, Shackley D, O'Reilly P. The community-based morbidity of flexible cystoscopy. BJU Int. 2002; 89, 347–349.

[13] Karakiewicz P, Benayoun S, Zippe C, Ludecke G, Boman H, Sanchez-Carbayo M, Casella R, Mian C, Friedrich M, Eissa S et al. Institutional variability in the accuracy of urinary cytology for predicting recurrence of transitional cell carcinoma of the bladder. BJU Int. 2006; 97, 997–1001.

[14] Raitanen M, Aine R, Rintala E, Kallio J, Rajala P, Juusela H, Tammela T, FinnBladder Group. Differences Between Local and Review Urinary Cytology in Diagnosis of Bladder Cancer. An

Interobserver Multicenter Analysis. Eur. Urol. 2002; 41, 284–289.
[15] Soukup V, Čapoun O, Cohen D, Hernández V, Burger M, Compérat, E, Gontero P, Lam T, Mostafid A, Palou J et al. Risk Stratification Tools and Prognostic Models in Non–muscle-invasive Bladder Cancer: A Critical Assessment from the European Association of Urology Non-muscle-invasive Bladder Cancer Guidelines Panel. Eur Urol. 2020; 6, 479–489.
[16] Hollenbeck B, Dunn R, Hollingsworth J, Skolarus T, Kim S, Montie J, Wood D, Miller D, Delays in diagnosis and bladder cancer mortality. Cancer. 2010; 116, 5235–5242.
[17] Liu Y, Wang X, Yang X, Wu X, He G. Pooled analysis of Xpert Bladder Cancer based on the 5 mRNAs for rapid diagnosis of bladder carcinoma. World J. Surg. Oncol. 2021; 19, 42.
[18] Babjuk, M, Burger M, Capoun O, Cohen D, Compérat E, Escrig J, Gontero P, Liedberg F, Lecomte M, Mostafid H, Palou J, Rhijn, Rouprêt M, Shariat F, Seisen, Soukup M. European Association of Urology Guidelines on Non–muscle-invasive Bladder Cancer (Ta, T1, and Carcinoma in Situ). European Urology.2022; 81(1), 75-94.
[19] Lotan Y, Black P, Caba L, Chang S, Cookson M, Daneshmand S, Kamat A, McKiernan J, Pruthi R, Ritch C, et al. Optimal Trial Design for Studying Urinary Markers in Bladder Cancer: A Collaborative Review. Eur. Urol. Oncol. 2018; 1, 223–230.
[20] Gontero P, Soukup V, Čapoun O, Cohen D, Hernández V, Burger M, Compérat E, , Lam T, Mostafid A, Palou J et al. Risk Stratification Tools and Prognostic Models in Non–muscle-invasive Bladder Cancer: A Critical Assessment from the European Association of Urology Non-muscle-invasive Bladder Cancer Guidelines Panel. Eur Urol. 2020; 6, 479–489.
[21] Koya M, Osborne S, Chemasle C, Porten S, Schuckman A, Kennedy A. An evaluation of the real world use and clinical utility of the Cxbladder Monitor assay in the follow-up of patients previously treated for bladder cancer. BMC Urol. 2020; 20, 12.
[22] Palou J, Brausi M and Catto JWF: Management of patients with normal cystoscopy but positive cytology or urine markers. Eur Urol Oncol. 2019.
[23] Machida Y, Hamlin J, Dutta A.Right place, right time, and only once: replication initiation in metazoans. Cell. 2005; 123: 13–24.
[24] Blow JJ, Gillespie PJ. Replication licensing and cancer–a fatal entanglement. Nat Rev Cancer.2008; 8: 799–806.
[25] Williams G, Romanowski P, Morris L, Madine M, Mills AD, et al. Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. Proc Natl Acad Sci.1995; 95: 14932–14937.
[26] Stoeber K. Diagnosis of genito-urinary tract cancer by detection of minichromosome maintenance 5 protein in urine sediments. Cancerspectrum Knowl Environ. 2002; 94: 1071.
[27] Burger M, Denzinger S, Hartmann A, Wieland WF, Stoehr R, et al. Mcm2 predicts recurrence hazard in stage Ta/T1 bladder cancer more accurately than CK20, Ki67 and histological grade. Br J Cancer. 2007. 96: 1711–1715.
[28] Dudderidge T, Stockley J, Nabi G et al: A novel, non-invasive test enabling bladder cancer detection in urine sediment of patients presenting with haematuriada prospective multicentre performance evaluation of ADXBLADDER. Eur Urol Oncol. 2020; 3: 42.
[29] Mertens L, Neuzillet Y, Horenblas S. et al: Landmarks in non-muscle-invasive bladder cancer. Nat Rev Urol. 2014; 11, 476–480.
[30] Amin M, Greene F, Edge S, Compton C, Gershenwald J, Brookland R, Meyer L, Gress D, Byrd D, Winchester D. The Eighth Edition AJCC Cancer Staging Manual: Continuing to Build a Bridge from a Population-Based to a More "Personalized" Approach to Cancer Staging. CA Cancer J. Clin. 2017;67:93–99.
[31] Levesque R. SPSS Programming and Data Management. A Guide for SPSS and SAS Users, Fourth Edition, SPSS Inc., 2007, Chicago, 3.
[32] Andersson E, Steven K and Guldberg P: Sizebased enrichment of exfoliated tumor cells in urine increases the sensitivity for DNA-based detection of bladder cancer. PLoS One. 2014; 9: e94023.
[33] Yu S, Wang Y, Chang J, Shen W, Chen H, Chiang C. Increased expression of MCM5 is significantly associated with aggressive progression and poor prognosis of oral squamous cell carcinoma. J Oral Pathol Med. 2014;43:344–349.
[34] Linton K, Rosario D, Thomas F et al: Disease specific mortality in patients with low risk bladder cancer and the impact of cystoscopic surveillance. J Urol. 2013; 189: 828.