Recurrence monitoring in Egyptian Bladder Cancer patients using effective and predictive urinary molecular biomarkers: A pilot study

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Abstract

Objective: In Egypt, Urinary bladder cancer (UBC) ranks the 3rd most common cancer, being the second among males and the 7th among females. One relevant mechanism to decrease mortality rate of UBC is to detect its local recurrence through using commercially available effective molecular biomarkers.

Purpose: We evaluate the diagnostic sensitivity and specificity of the Xpert BC Monitor test in Egyptian patients with non muscle invasive BC.

Methodology: Liquid biopsies in the form of voided urinary samples were analyzed using the Xpert BC Monitor Kit, measuring the levels of five target mRNAs (ABL1, CRH, IGF2, UPK1B, ANXA10) by RT-PCR in 20 Egyptian patients.

Results: Two patients (10%) were females and 18 (90%) were males with median age of 66 years. About 80% of the urine samples that were tested in this pilot study were successfully analyzed. Six patients (30%) had positive test results, while 10 patients (50%) had negative test results, 6 of them had radiological evidence of disease. Invalid test results were found in 4 patients (20%). Thus, the test showed 60% sensitivity with 100% specificity and giving positive predictive value of 100% with negative predictive value of 60%.

Conclusion: Using the non-invasive liquid biopsy method represents a promising tool to improve the current standard of care in patients with non muscle invasive bladder cancer. Further prospective randomized trials with larger patient series and longer follow up period are needed to establish the superiority of Xpert BC Monitor test over the current standard of care in Egyptian Cancer patients.

Keywords:Urinary bladder cancer, recurrence rate, urinary molecular biomarkers.

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Introduction

Urinary bladder cancer (UBC) represents the ninth most common malignancy worldwide, being the seventh commonest cancer in males and the seventeenth in females.[1]In Egypt, UBC ranks the 3rd most common cancer, being the second among males and the 7th among females, with age standardized ratios of 21.1 and 5.5 per 100,000/year in either sex respectively.[2] Egyptian males have the highest mortality rates (16.3 per 100,000) worldwide, which is twice as high as the highest rates in Europe and over 4 times higher than that in the United States. One relevant mechanism to decrease mortality rate of UBC is to early detect its local recurrence. Accordingly, emergence of commercially available effective and predictive urinary molecular biomarkers had been introduced for detecting UBC recurrence; which were considered to reduce or even replace follow-up cystoscopies and cytologies.[3]Xpert Bladder Cancer Monitor is an mRNA-based urinary marker test which designed to measures the levels of five target mRNAs (ABL1, ANXA10, UPK1B, CRH, andIGF2) and provide up-to-date information on BC recurrence, using a noninvasive urine specimen. Annexin A10 (ANXA10) is one of the markers which predict progression in early and advanced UBC stages. Additionally, ANXA10 is responsible for regulating cell growth and cell migration.[4] Uroplakin Ib is a structural protein on the urothelial cells. Levels of uroplakin Ib mRNA are definitely reduced or even absent in many transitional cell carcinomas, but the molecular mechanisms responsible is not yet clear. A known mechanism involved in silencing gene expression in tumors is hypermethylation of CpG islands within the 5` promoter regions of genes.[5] The Corticotropin Releasing Hormone (CRH)-system has important role in human malignancies development through migration of tumor cells.[6] Insulin-like growth factor (IGF) pathways are considered as probable molecular targets for cancers treatment. Insulin-like growth factor 2 (IGF2) is demonstrated to be potent mitogen and have growth-promoting activity. Abnormal IGF2 expression or dysfunction of IGF2 signaling is described in many types of cancers.[7]

Clinical significance

Despite the fact that Egyptian UBC males have the highest mortality rates worldwide, new non invasive biomarkers have emerged to decrease this mortality rates & to detect its early local recurrence. The aim of the present pilot study was to evaluate the diagnostic sensitivity and specificity of the Xpert BC Monitor test in the Egyptian patients with non muscle invasive UBC.

Subjects and methods

Study population:

The present study included 20 Egyptian patients previously diagnosed with non muscle invasive urinary bladder transitional cell carcinoma (TCC). Patients were recruited randomly from the outpatient clinic at Kasr Al-Ainy Center of Clinical Oncology and Nuclear Medicine between June 2017 and December 2017 (fig.1). This study was approved by Kasr Al-Ainy Center of Clinical Oncology and Nuclear Medicine Institutional Review Board (IRB).

Sample collection, preparation and RT-PCR methodology:

Voided urinary samples were analysed using the Xpert BC Monitor Kit (Cepheid, Sunnyvale, CA, USA), on the Cepheid GeneXpert® Dx System. This test is automated, quantitative RT-qPCR which measures the levels of five target mRNAs (ABL1, CRH, IGF2, UPK1B, ANXA10) by RT-PCR according to the manufacturer`s protocol. Transfer 4.5 ml of voided urine specimen to Xpert transport tube within one hour of collection and invert three times to mix. The transfer pipette provided with Xpert Bladder Cancer Monitor is used to transfer 4 mL of treated urine to the Sample Chamber of the cartridge. All reagents required for sample preparation and RT-PCR analysis are preloaded in the cartridge. Cells in the urine sample are captured on a filter and lysed by sonication. The released nucleic acid is eluted, mixed with dry RT-PCR reagents, and the solution is transferred

to the reaction tube for RT-PCR detection. Time to result is approximately 90 minutes. ABL1 serves as a Sample Adequacy Control (SAC). The ABL1 ensures that the sample contains human cells and human RNA. A positive ABL1 signal is required for a valid test result. A Probe Check Control (PCC) is included to verify reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. A Cepheid Internal Control (CIC), designed to detect sample-associated inhibition of the real-time RT-PCR, is included in each cartridge. Xpert Bladder Cancer Monitor provides positive or negative test results based on the results of a linear discriminant analysis (LDA) algorithm that utilizes the cycle threshold (Ct) results of the five mRNA targets. The LDA Total must be within the valid range of -20 to 20. It is not necessary to detect all of the mRNA targets for a positive test result, table (1).

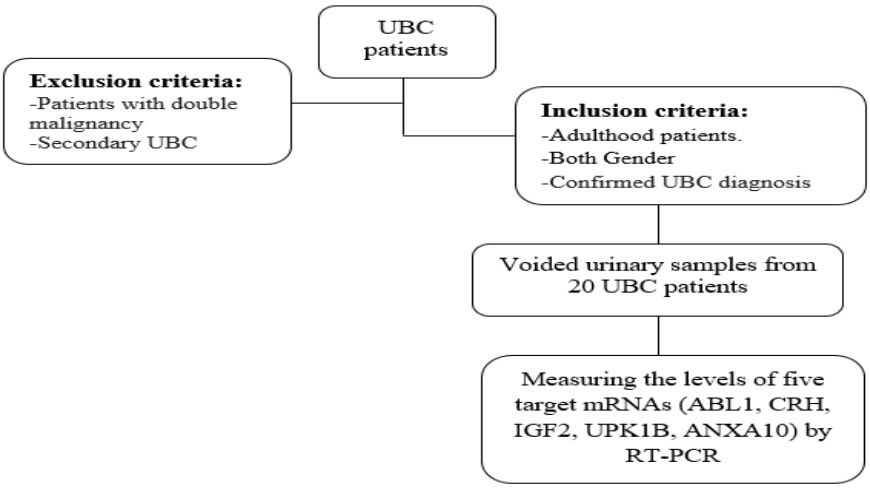

Figure (1): Flow chart of studied UBC patients

Results:

Descriptive and histopathological characteristics of UBC patients:

Our study included 20 Egyptian patients diagnosed with non muscle invasive urinary bladder transitional cell carcinoma (TCC). Among the 20 UBC patients (Table 2), 2 (10%) were females and 18 (90%) were males with a female to male ratio 0.11: 1. Their ages ranged from 48 to 83 years with mean ± SD of 66.78 ± 11.04 years & median of 66 years. Eight patients (40%) had tumor size < 3 cm, while 12 patients (60%) had tumor size ≥3 cm. Ten patients (50%) had tumor stage; T2, while T3 & T4 accounts for 40% & 10% of the BC patients, respectively. Only 20% of the patients had grade II BC, while the rest were grade III.

Urinary molecular biomarkers:

Over 80% of the urine samples that were tested in this study were successfully analyzed by Xpert BC Monitor Kit. Six patients (30%) had positive test results while 10 patients (50%) had negative test results with 6 of them had radiological evidence of disease. Invalid test results were found in 4 patients (20%). Thus, the test showed 60% sensitivity with 100% specificity and giving positive predictive value of 100% with negative predictive value of 60%. Magnetic Resonance Imaging (MRI) was done to confirm the diagnosis of the six positive UBC patients and they were 4 males (66.7%) and 2 females (33.3%). They had average age of 66-80 years, mean ± SD of 72.33 ± 7.09 and median age of 71 years (> 60 years). Four of them (66.7%) had tumor size ≥3 cm as shown in table 3. All positive patients were grade III with 4 patients had tumor stage (T2), the remaining 2 were T3. Four positive patients were designated to receive palliative therapy, while the remaining 2 patients were designated for surgery.

Discussion:

Our study is the first study to test the sensitivity of Xpert BC Monitor Kit in Egyptian non muscle invasive UBC patients. Guidelines strongly recommend stratifying risk of recurrence and progression and using risk tables to determine appropriate treatment for bladder cancer patients.[8] After therapy and due to the high rate of recurrence in BC patients which approaches approximately 70%, patient follow-up consists of cystoscopies and cytologies. Cystoscopy is an invasive endoscopy of the bladder that requires urethral catherization, which may affect patients' adherence to follow-up.[9]Recently, liquid biopsy appears as non-invasive methods in the diagnosis and follow-up of cancer patients in comparison to tissue biopsywith testing of cell-free tumorderivativenucleic acids in patient's body fluidssuch as plasma, serum, broncho-alveolar lavage, stool, urine,etc.[10] To address this problem, Cepheid GeneXpert® Dx System analyzes urine samples to measure five target mRNAs (ABL1, CRH, IGF2, UPK1B, and ANXA10) that are non-invasive validated PCR-based assay of urine markers that could be an indicator of disease recurrence and reduce the number of cystoscopies in routine recurrence monitoring.[11] Few years earlier, Mengual et al., 2010 revealed urinary gene expression profile panel (ANXA10, CRH, MAGEA3, AHNAK2, KLF9, CTSE, KRT20, PPP1R14D, IGF2, POSTN, SLC1A6, and TERT + ASAM and MCM10) that had sensitivity of 80% for the detection of low-grade NMIBC and 93% for the detection of high-grade NMIBC.[12] Three genes (ANXA10, IGF2 and CRH) from the Mengual panel were included in the Xpert BC Monitor kit, which included in addition, ABL1 and UPK1B genes.[13] Our results using Xpert Bladder Cancer Monitor kit had an overall sensitivity of 75%, sensitivity for high grade tumors, specifically of 84%, and an overall specificity of 80.6%. The test has an overall 93.9% negative predictive value, providing urologists with actionable results, potentially reducing the need for frequent invasive testing and though, reducing the cost and patient discomfort. Our results were in accordance to Van Valenberg et al., 2019 who performed a study on validation of Xpert Bladder Cancer Monitor test in 239 NMIBC patients and revealed an overall sensitivity of 74% and 83% for high grade tumors with specificity of 80%. The overall NPV was 93% and 98% for high grade tumors.[14] In 2018, Pichler et al., reported an overall sensitivity of 84%, NPV of 93% in the Xpert BC Monitor kit with specificity of 91% and sensitivity of 77% in low grade BCs with all high grade tumors were detected by this kit.[15] Also, Van Valenberg et al., 2017 showed overall sensitivity, specificity and NPV of 75%, 81% and 94%, respectively when testing the effect of the Xpert BC Monitor kit in surveillance of 255 BC patients.[16] The test requires less than 2 min of hands-on sample preparation time and it delivers results in approximately 90 minutes, which may improve compliance in patients being monitored for bladder cancer recurrence. It also, improves workflow management for urologists and their bladder cancer patients because it provides results within the same day that inform important downstream management decisions.

In conclusion: Using the non-invasive liquid biopsy method represents a promising tool to improve the current standard of care in patients with non muscle invasive bladder cancer. In addition, it has the advantage of easy handling, short hands-on sample preparation time and results delivered within the same day; however, the cost of the kit is a crucial issue to be cost effective in comparison to other techniques.Further prospective randomized trials with larger patient series and longer follow up period are needed to establish the superiority of Xpert BC Monitor kit over the current standard of care in BC monitoring & follow up.

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Conflicts of interest: Authors declared no conflicts of interest.

Ethical approval: All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments (GCP guidelines) or comparable ethical standards.

Informed consent was obtained from all participants included in the study.

Table 1: Xpert Bladder Cancer Monitor Representative Results and Interpretation

Result Interpretation	**Result Interpretation**
Positive	•The LDA Total (the result of an algorithm that uses the Ct values of ABL1, ANXA10, UPK1B, CRH and IGF2) is equal to, or above the cut off. • The LDA Total must be within the valid range of -20 to 20. • ABL1: ABL1 Ct is within the valid range. • CIC: Not applicable. The CIC results are ignored because the assay targets in positive samples can interfere with this control. • PCC– PASS; all probe check results pass
Negative	• The LDA Total is below the cut off. • ABL1: ABL1 Ct is within the valid range. • CIC: CIC Ct is within the valid range. • PCC– PASS; all probe check results pass
Invalid	Presence or absence of target mRNAs cannot be determined. • ABL1 and CIC: ABL1 Ct and /or CIC Ct do not meet acceptance criteria or one or more of the growth curves do not meet acceptance criteria. • PCC– PASS; all probe check results pass. • The cellular content in the sample is too low, PCR was inhibited or the sample was not properly collected.
Error	Presence or absence of target mRNAs cannot be determined. • PCC FAIL; all or one of the probe check results fail. • Possible reasons for error include the reaction tube was filled improperly, a reagent probe integrity problem was detected, pressure limits were exceeded, or a valve position error was detected.
No result	Presence or absence of target mRNAs cannot be determined. • A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • PCC– NA (not applicable)

Table 2: Patients` characteristics

Characteristics	**No = 20** (%)
Gender:	
Males	18 (90%)
Females	2 (10%)
Age (ys):	
Range	48-83
Mean ± SD	66.78 ± 11.04
Median	66
Number of tumours	
Single	20 (100%)

Characteristics	**No = 20** (%)
Number of recurrences	
0	20 (100%)
Tumour size	
<3 cm	8 (40%)
≥3 cm	12 (60%)
Stage	
T2	10 (50%)
T3	8 (40%)
T4	2 (10%)
Grade	
II	4 (20%)
III	16 (80%)

Table 3: Positiveurinary molecular biomarkers patients` characteristics

Characteristics	**No = 6/20** (30%)
Gender:	
Males	4/6 (66.7%)
Females	2/6 (33.3%)
Age (ys):	
Range	66-80
Mean ± SD	72.33 ± 7.09
Median	71
Tumour size	
<3 cm	2/6 (33.3%)
≥3 cm	4/6 (66.7%)
Stage	
T2	4/6 (66.7%)
T3	2/6 (33.3%)
Grade	
III	6/6 (100%)

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مراقبة معدل الانتكاس فى مرضى سرطان المثانة المصريين باستخدام المؤشرات الجزيئية البولية الفعالة والتنبؤية: (دراسة تجريبية)

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الملخص

الخلفية: يصنف سرطان المثانة بمصر في المرتبة الثالثة بين السرطانات الأكثر شيوعًا، والثاني بين الذكور والسابع بين الإناث. وتتمثل إحدى الآليات ذات الصلة لتقليل معدل وفيات سرطان المثانه في اكتشاف معدل الانتكاس المحلي من خلال استخدام المؤشرات الحيوية الجزيئية الفعالة المتاحة تجارياً.

الأهداف: تقوم الدراسة بتقييم حساسية التشخيص، واختبار قياس المؤشرات الحيوية الجزيئية في المرضى المصريين الذين يعانون من سرطان المثانه غير المنتشر للعضلات.

الطريقة: تم تحليل عينات بولية لعشرين مريضا مصريا لقياس مستويات خمسة من المؤشرات الحيوية الجزيئيةABL1) ، CRH، IGF2UPK1B، (ANXA10بواسطة تحليل تفاعل البلمرة المتسلسل فى الوقت المحدد.

النتائج: يوجد فقط مريضان (10٪) من الإناث و18 (90٪) من الذكور بمتوسط عمر 66 سنة، وتم تحليل حوالي 80٪ من عينات البول التي تم اختبارها في هذه الدراسة التجريبية بنجاح، وستة مرضى (30٪) كانت لديهم نتائج اختبار إيجابية، وفي حين أن 10 مرضى (50٪) كانت نتائج اختباراتهم سلبية، 6 منهم كانت لديهم أدلة إشعاعية على المرض،وتم العثور على نتائج اختبار غير صحيحة في 4 مرضى (20٪). وأظهر الاختبار حساسية 60٪ مع خصوصية 100٪ وإعطاء قيمة تنبؤية إيجابية 100٪ مع قيمة تنبؤية سلبية 60٪.

الاستنتاج: يمثل استخدام طريقة الخزعة السائلة غير الغازية أداة واعدة لتحسين المستوى الحالي للرعاية في المرضى الذين يعانون من سرطان المثانة غير المنتشر للعضلات، وهناك حاجة إلى مزيد من التجارب العشوائية المرتقبة مع سلسلة أكبر من المرضى، ومدة متابعة أطول لإثبات تفوق اختبار قياس المؤشرات الحيوية الجزيئية على المعيار الحالي للرعاية في مرضى سرطان المثانه المصريين.

الكلمات الدالة:سرطان المثانة البولية، معدل الانتكاس، المؤشرات الحيوية الجزيئية البولية.