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Evaluation of Gene Expression Level of miRNA-29c, miRNA-125, miRNA-141, miRNA-145 and miRNA-205 as Predisposing Factors for Transitional Cell Carcinoma-Bladder Cancer in Iraqi Patients

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Abstract

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally. Transitional cell carcinoma(TCC) of the bladder Cancer (C) account 95 percent of bladder malignancies, with males having a greater prevalence than females. The current study sought to determine whether there is a link between miRNA-29c, miRNA-125, miRNA-141, miRNA-145 and miRNA205 expression levels and TCC/BC risk in Iraqi bladder cancer patients. In the current prospective cross-sectional investigation, 149 samples were collected (95 urine and 54 tissue biopsies). From November 2018 to August 2019, 37/95 urine samples were randomly taken from healthy persons. Total RNA was extracted from tissue and urine samples, and then converted to cDNA via reverse transcription. Quantitate Real-Time-PCR was done using specific primers for quantification of gene expression level of the studied miRNAs. The results showed that 32/49 (65%) patients had non-muscles invasive bladder cancer (stage T1), while 17/49 (35%) patients had muscles invasive bladder cancer (stage T2-T4). Fold change in miRNA-125 expression level showed highly significant differences between non-muscles invasive bladder cancer (T1) and muscles invasion bladder cancer (T2-T4) in both urine and tissue samples biopsies using Chi-square test at $p \leq 0.01$. Whereas miR-29c, miR-141, miR-145 and miR-205 showed no significant differences at $p > 0.05$ between muscles invasive bladder cancer (T1) and muscles invasive bladder cancer (T2-T4) in both urine and tissue biopsies samples. Thus, miR-125 can be associated with the development of invasive stages of TCC-BC as there is an increase in miR-125 expression level in the urine of patients during the final stage of cancer.Hence, this gene could be considered a good predictor in advanced stages of bladder cancer.

Key word: MicroRNAs, transitional cell carcinoma, bladder cancer, prognosis biomarker.

تقييم مستوى التعبير الجيني للحمض النووي الريبوزي الميكروي (miRNA-29c و miRNA-125 و miRNA-141 و miRNA-145 و miRNA-205) كعوامل تنبؤ لحدوث سرطان الخلايا الانتقالية في المثانة لدى المرضى العراقيين

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الخلاصة

الحمض الريبوزي النووي الميكروي (microRNA) هو حمض نووري ريبوزي صغير لايشفر الى بروتين وانما ينظم التعبير الجيني بعد عملية الاستنساخ ، سرطان الخلايا الانتقالية للمثانة يشكل نسبة (95%) من انواع سرطان المثانة الاخرى وبنسب اصابة عند الذكور اعلى من الاناث. تهدف الدراسة الحالية الى التحقق فيما اذا كان هناك ارتباط بين مستوى التعبير الجيني للحمض الريبوزي الميكروي miRNA−29c و miRNA−125 و miRNA−141 وmiRNA−145 وmiRNA−205 والاستعداد للاصابة بسرطان الخلايا الانتقالية للمثانة لدى المرضى العراقيين. ضمت الدراسة الحالية 149 عينة (95/ 149ادرار و 149/54نسيج) تضمنت 95/37 عينة ادرار تم جمعها عشوائيًا من أشخاص أصحاء، تم جمع العينات من تشرين الثاني 2018 إلى اب 2019 . تم استخلاص إجمالي الحمض النووي الريبوزي من عينات الادرار والنسيج وحولت الى cDNA. تم إجراء التقدير الكمي بواسطة Real time−PCR باستخدام بادئات محددة لتقدير مستوى التعبير الجيني للـ miRNAs المدروسة. أظهرت النتائج ان 49/32 (65%) من مرضى سرطان الخلايا الانتقالية للمثانة من نوع غير مخترق للعضلات (مرحلة T1) بينما 32/17 (35%) من النوع المخترق للعضلات (مرحلة من T2 الى T4). التغيير الكمي miR−125 اظهر فروق معنوية عالية بالتعبير الجيني عند تحليل البيانات باستخدام مربع كاي وبمستوى معنوية $p \leq 0.01$ بين مرضى سرطان الخلايا الانتقالية للمثانة من نوع غير المخترق للعضلات (مرحلة T1) والنوع المخترق للعضلات (مرحلة من T2 الى T4) في كل من عينة الادرار والنسيج، بينما الانواع الاخرى miR−29وmiR141 و miR145 و miR205 لم تظهر فروقا معنوية على مستوى دلالة $p > 0.05$ بين مرضى سرطان الخلايا الانتقالية للمثانة من نوع غير مخترق للعضلات (مرحلة T1) والنوع المخترق للعضلات (مرحلة من T2 الى T4) في كل من عينات الادرار والنسيج. لذا من الممكن ان يكونmiR−125له علاقة بتطور المراحل المتأخرة من سرطان الخلايا الانتقالية للمثانة، ونظرا لزيادة التعبير الجيني لmiR−125 في إدرار مرضى سرطان المثانة في المراحل المتطورة، فأن من الممكن إن نعتبرهذا الجين أداة تنبؤ جيدة في المرحلة المتطورة من سرطان المثانة.

1. Introduction

Bladder Cancer (BC) is the fourth most common cancer among the headmost ten malignancies of the urinary tract, that leads to death [1],with the incidence being four times higher in men than in women [2]. More than 90% of BCs form in the epithelial cell lining of the bladder (the urothelium) and are known as urothelial carcinoma or transitional cell carcinoma (TCC) [3]. TCC/BC tumorigenesis and its progression are aided by an unknown mechanism that includes irreversible genetic and reversible epigenetic alterations, chromosomal abnormalities and genetic polymorphisms [4]. Many evidences increasingly point to specific genetic and epigenetic alteration patterns in BC, even between different stages and grades of cancer [5]. MiRNAs are short, single-stranded RNA molecules, about 22 nucleotides in length, an epigenetic mechanism, are endogenous non-coding protein. RNA molecules negatively modulate gene expression at the post-transcriptional level in a sequence specific manner [6]. They act by binding to complementary sequences in the 3untranslated regions of specific mRNAs, resulting in the inhibition of translation [7]. Each miRNA has multiple targets Hence, changes in the profile of expressed miRNAs can have multiple effects on cellular phenotype [8]. MiRNAs target cell mechanisms, such as apoptosis, cell cycle, and epithelial–

mesenchymal transition (EMT), allowing cancer cells to escape cell death [5]. MiR-29c regulates the apoptotic protein MCL1 and thereby regulates apoptosis as well as DNA de novo methyl transferases DNMT3A and DNMT3B, key enzymes that are frequently upregulated in cancer [9]. Dysregulation of miR-125b is related to tumorigenesis, cancer progression, and metastasis [10]. MiR-145 inhibits cell proliferation and migration through direct regulation of the actin-binding protein, fascin in BC [11]. The miR-200 family, including miR-141, targets the oncogene transcription factor 3(E2F3) which is critical for the G1/S transition and is over expressed in most high-grade BCs [12]. Down regulation of miR-205-5p has been linked to the epithelial-mesenchymal transition (EMT) and has also been significantly associated with progression in non-muscle invasive BC [13]. The current study aimed to investigate if there was any association between expression levels of miRNA29c, miRNA-125, miRNA-141, miRNA-145 and miRNA-205 and predisposing to TCC/BC in a set of Iraqi patients.

2. Materials and Methods

The current cross-sectional study included 149 samples (95 urine and 54 tissue) collected from AL-Imamein AL-Kadhmain Medical City Hospital and Ghazi-AL-Hariri Specialized Surgery Hospital, Medical City Hospital, Baghdad, Iraq during period extending from November 2018 to August 2019. From the urine samples, 37/95 were randomly collected from healthy individuals. Patients included in the present study were from different provinces in Iraq. Classification of subjects and samples were done according to histopathological findings and cystoscopy as shown in Table 1.

Table 1: Classification of subjects and samples included in current study according to histopathological findings and cystoscopy

Subject group	Histo-pathological findings and cystoscopy	*No*. of subject	*No*. of collected urine sample	*No*. of collected tissue biopsy	Total *No*.
Patient with TCC-BC*	Non-invasiveTCC-BC (Ta and T1)	32	27	31	89
	Invasive TCC-BC (T2, T3 and T4)	17	16	15	
Negative control group for BC	Patients with negative cystoscopy**	12	12***	4	16
	Patients with another type of cancer (adenocarcinoma and prostate cancer)	4	3	4	7
	Healthy individual	37	37	-	37

No: represent the number, * Patient with transitional cell carcinoma in different stages, **Patient with negative cystoscopy (have benign mass or have not mass -no pathological changes in bladder tissue), ***Urine samples were obtained from those 12 patients and in 4 patients, tissue biopsy samples were obtained in addition to urine samples.

TCC/BC classification was done according to cystoscopy findings, which was either high or low grade according to the World Health Organization (WHO) classification criteria 2018[14].

Tissue biopsy and urine samples from included subjects were kept at -20°C for molecular analysis. Data collected from each patient and the control group included name, age, gender, smoking and previous treatment in patients with recurrence of TCC/BC. Histopathology findings of each patient were obtained from the laboratory reports.

Inclusion criteria: Patients with bladder tumor and patients with negative cystoscope (benign tumor or no histopathological changes).

Exclusion criteria: Patient treated with chemotherapy, immunotherapy or antibiotics for UTIs for less than 1 month. This study was conducted in accordance with the Declaration of Helsinki (1964) and was approved by Ethical Committee of the College of Medicine-AL-Nahrain University.

2.1 Extraction of miRNA from urine

Following manufacturer instructions, total RNA was extracted from urine by using miRNeasy Mini kit (QIAGEN ® Cat. No.217004, Germany). Before starting the procedure, urine was concentrated by centrifugation at 1000xg for 5 min. Then, the supernatant was dispensed and the rest of urine was added to the sediments and re-centrifuged. The step was repeated until all urine samples were centrifuged. The differences in procedure of miRNA extraction from tissue biopsy usingmiRNA extraction procedure from urine was that in the first step, tissue biopsy was chopped into very tiny clumps using sterile scissors in order to gain free cells. Purity and concentration of extracted RNA were estimated using Nanodrop spectrophotometer at 260nm and 280nm absorption wavelengths.

2.2 Reverse transcription of miRNA

Following manufacturer instructions, cDNA synthesis from template miRNA was done by using miScript II RT Kit (QIAGEN® Cat.No218161, Germany). Purity and concentration of extracted cDNA was estimated using Nano drop spectrophotometer at 260/280 absorption wavelengths.

2.3 Quantities Real-Time-Reverse Transcription-PCR

Real-Time-Reverse Transcription-PCR(RT-RT-PCR) was done for the diluted cDNA by using miScript SYBR® Green PCR Kit (Qiagen-Cat.No. 18075, USA) and miScript Primer assay for quantification of hsa-miR-29c-3p, hsa-miR-125a-5p, hsa-miR-141-3p, hsa-miR-145-5p, hsa-miR205-5p. Specific primer for SNORD61 was used to amplify housekeeping gene. Master mix was prepared (per one reaction) by mixing 2.5 µl of QuantiTect SYBR Green PCR Master Mix(2x),2.5 µl of 10XmiScript Universal Primer, 2.5 µl of 10XmiScript Primer Assay and RNase-free water until 22.5 µl. Then,2.5 µl of cDNA with final concentration of 75ng/reaction was added to the reaction tube. No template control (NTC) tube was prepared that contained all PCR master mix components.Instead of cDNA, 2.5 µl of nuclease free water was added. Reaction tubes were placed in Real-Time thermal cycler which was programmed as shown in Table 2. Relative method was used to calculate fold changes of gene expression level [15].

Table 2: Cycling conditions for RT-PCR.

Step	Time	Temperature	Additional comments
PCR Initial activation step	15 min	95ºC	HotStarTaq DNA Polymerase is activated by this heating step
		Cycling condition	
Denaturation	15s	94 ºC	
Annealing	30s	55 ºC	
Extension	34s	70 ºC	Perform fluorescence data collection
		Cycle number	40 cycles

2.4 Statistical Analysis

Data was summarized, analysed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Quantitative variables were expressed as mean and standard deviation (SD). Whereas, categorical variables were expressed as numbers

and percentages. Chi-square test was used to study association between any two categorical variables.However, Yates correction was used instead when more than 20% of cells had expected count less than 5 and Fisher exact test when a cell or more contained an observed value of zero. The level of significance was set at $P \leq 0.05$.

3. Results

General demographic criteria used in this study included gender, smoking and bacterial infection of patients with TCC/BC and patients negative for TCC/BC as illustrated in Figure 1. Age range of patients with TCC/BC was 32-87 years and of patients negative to TCC/BC (including healthy individuals, patients with negative cystoscopy and patients with adenocarcinoma and prostate cancer) was 4-62 years. Male to female percentages of patients with TCC/BC were 38/49 (77.5%) and 11/49 (22.5%), respectively. Male to female ratio of patients with TCC/BC was 3.4:1 (38:11). The age of patients with negative cystoscopy ranged from 16 years to 76 years. For patients with TCC/BC, the highest percentage of 69% (34/49) was seen in age between 55-88 years, while it was 30%, (15/49) in age group younger than 55 years.

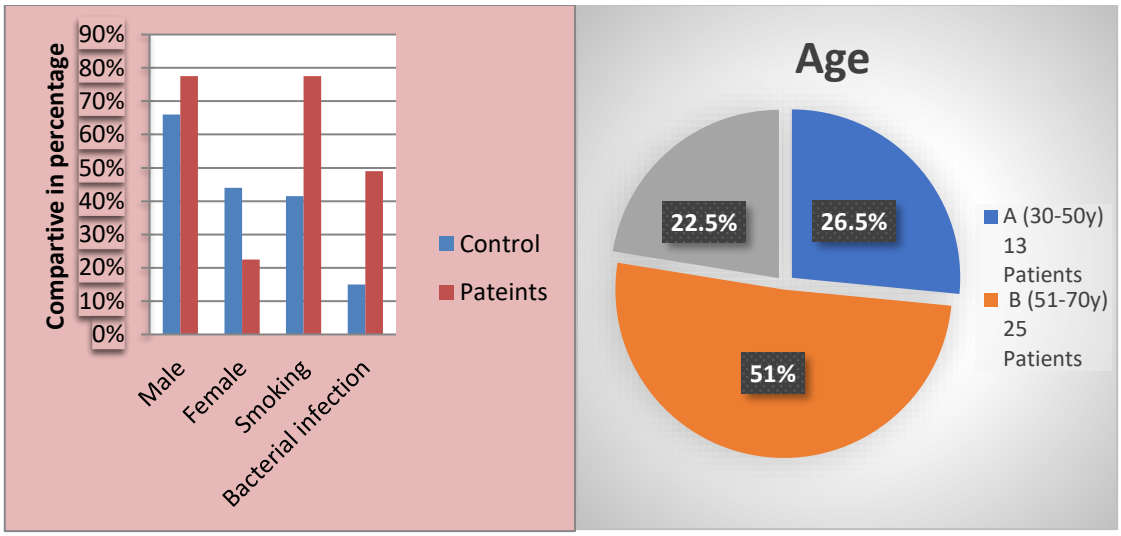

Figure 1: Left: Comparing between patient with TCC/BC and patients negative for TCC-BC in sex, smoking and bacterial infections. Right: Distribution of patients with TCC/BC among age groups.

3.1 Histo-pathological findings

The histo-pathological findings of 62 tissue biopsies samples showed that 49/62 (79.03%) were diagnosed as TCC/BC, 1/62 (1.61%) sample was diagnosed as adenocarcinoma and 12/62 (19.35%) samples showed normal histopathology related to patients negative to cystoscope who had symptoms of TCC/BC or had missed diagnosis as TCC/BC using routine methods of diagnosis, but cystoscopic surgery showed no tumor or benign tumor. From the total of 49 patients with TCC-BC, 39 suffered from TCC/BC for first time while 10 patients had TCC/BC for the second or third time within one year or less (recurring cancer). Patients with TCC/BC (without recurrence) (39/49) were classified as low-grade tumor or non-muscle invasive-NMI (pTa and pT1) which constituted the majority, 25/39 (64.1%) of patients. High-grade tumors or muscle-invasive cancers (MIBC) (T2, T3, T4) were diagnosed in 14/39 (35.9%) patients. In patients with recurring TCC-BC, 7/10 (70%) patients were diagnosed with low-grade tumors while 3/10 (30%) patients with high-grade tumors (Table 3). The recurrence rate from total

TCC-BC cases was 10/49 (20%), since the recurrence rate following the trans-urethral resection of bladder tumor (TURBT).

Table 3: Distribution of patients with different stages of bladder cancer according to recurrence

Stage Patients	T1 *No.* (%)	T2 *No.* (%)	T3 *No.* (%)	T4 *No.* (%)
Patients with TCC-BC without recurrence	25/39 (64.1%)	9/39 (23%)	3/39 (7.6%)	2/39 (5.1%)
Patients with recurrent TCC-BC	7/10 (70 %)	2/10 (20%)	-	1/10 (10%)
Total	49 patients			

No: represent the number, NMI (pTa and pT1): low-grade tumor or non-muscle invasive. (MIBC) (T2, T3, T4): high-grade tumors or Muscle-invasive cancers.

3.2 The expression level of the studied miRNAs in urine and tissue biopsies samples in correlation with histo-pathological findings

Regarding miR-29c, up-regulation in gene expression level in urine samples from patients with different stages of TCC/BC was seen in 25/41 (60.9%) patients, while 16/41(39.1%) samples showed down-regulation. Up-regulation in gene expression level in tissue biopsies samples from patients with different stages of TCC/BC was seen in 27/43 (62.7%) patients, whereas 16/43 (37.3%) patients had down-regulation. Fold changes in gene expression level of studied miRNAs in urine and tissue biopsies samples from patients with TCC/BC is shown in Table 4.

Table 4: Fold change in gene expression level of studied miRNAs in patients with TCC-BC in urine and tissue biopsies samples.

Patients with TCC-BC		Total No.=43*	Up regulation of gene expression level		Down regulation of gene expression level	
miRNA			*No.*	%	*No.*	%
miR-29c	Urine	41	25	60.9	16	39.1
	Tissue	43	27	62.7	16	37.3
miR-125	Urine	41	23	56	18	44
	Tissue	43	23	53.4	20	46.6
miR-141	Urine	41	15	36.5	26	63.5
	Tissue	43	19	44.1	24	55.9
miR-145	Urine	41	21	51.2	20	49.8
	Tissue	43	27	62.7	16	37.3
miR-205	Urine	41	22	53.6	19	46.4
	Tissue	43	24	55.8	19	44.2

No: represent the number**,** *Not all patient had urine and tissue biopsies samples (3 patients had only urine sample and 6 patients had only tissue biopsies samples).

Fold changes for miRNA-125 showed highly significant differences using Chi-square test at $p \leq 0.01$ in gene expression level between non-muscles invasive bladder cancer (T1) and muscles invasion bladder cancer (T2-T4), in both urine and tissue biopsies samples. Whereas miR-29c, miR-141, miR-145 and miR-205 showed no significant differences at $p > 0.05$ between muscles invasive bladder cancer (T1) and muscles invasive bladder cancer (T2-T4), in both urine and tissue biopsies samples (Table 5).

Table 5: Comparison between non-muscles invasive (T1) and muscles invasion bladder cancer (T2-T4)

Patients with TCC-BC	Non-muscle Invasion	Muscles Invasion	*p*

miRNA		Up-regulation *No*. (%)	Down-regulation *No*. (%)	Up-regulation *No*. (%)	Down-regulation *No*. (%)	
miR-29c	Urine	15/25 (60%)	10/25 (40%)	9/16 (56%)	7/16 (44%)	0.812 C NS
	Tissue	19/29 (65.5%)	10/2 (34.5%)	8/14 (57%)	6/14 (43%)	0.653 C NS
total			54		30	
miR-125	Urine	10/25 (40%)	15/25 (60%)	13/16 (81%)	3/16 (19%)	0.009 C HS
	Tissue	18/29 (62%)	11/29 (38%)	3/14 (21%)	11/14 (79%)	0.009 C HS
total			54		30	
miR-141	Urine	9/25 (36%)	16/25 (64%)	6/16 (37.5%)	10/16 (62.5%)	0.923 C NS
	Tissue	16/29 (55%)	13/29 (45%)	5/14 (35.7%)	9/14 (64.7%)	0.190 C NS
total			54		30	
miR-145	Urine	10/25 (40%)	15/25 (60%)	9/16 (56%)	7/16 (44%)	0.309 C NS
	Tissue	19/29 (65.5%)	10/29 (34.5%)	7/14 (50%)	7/14 (50%)	0.261 C NS
total			54		30	
miR-205	Urine	13/25 (52%)	12/25 (48%)	10/16 (62.5%)	6/16 (37.5%)	0.509 C NS
	Tissue	19/29 (65.5%)	10/29 (34.5%)	5/14 (35.7%)	9/14 (64.7%)	0.079 C NS
total			54		30	

*N*o: represent the number, C: Chi-square test; NS: Not significant at $p > 0.05$; HS: Highly significant at $p \leq 0.01$

Fold changes in gene expression levels of miR-29c, miR-141 and miR-205 in urine samples from TCC/BC patients with recurrence showed down regulation in 7/8 (87.5%) patients, 6/8 (75%) patients and 6/8 (75%) patients respectively. Fold changes in gene expression levels of miR-125 in tissue biopsies samples showed down regulation in 4/5 (80%) patients. Compression of fold changes in gene expression level of studied miRNAs in urine and tissue samples between patients with TCC/BC (non-invasive TCC/BC, invasive TCC/BC and recurrent TCC/BC) and patients negative for TCC/BC (negative cystoscopy patients and patients with another type of cancer to TCC/BC) using fisher exact test and Chi square showed that there was no statistically significant changes in expression levels of miR-29c, miR-125, miR-141, miR145 and miR-205 in urine and tissue biopsies samples ($P>0.05$), in comparison between patients with TCC/BC and any other type of cancer. There was statistically significant difference in fold changes of gene expression level of miR-125 between urine and tissue biopsies samples from patients with invasive TCC/BC. Also, there was statistically significant difference in fold change of gene expression level of miR-205 between urine and tissue biopsies samples from patients with another type of cancer. Summary of comparison of fold change in gene expression level of studied miRNAs between urine and tissue biopsies samples among patients negative for TCC/BC and patients with TCC/B is shown in Table 6.

Table 6: The changes in gene expression level of studied miRNAs among studied subject groups

miRNA	Sample	Fold change in gene expression level *No.* (%)	No. of patients with Negative cystoscopy *No.* (%)	another types of cancer *No.* (%)	No. of patients with non-invasive TCC-BC *No.* (%)	No. of patients with invasive TCC-BC *No.* (%)	No. of patients with recurrent TCC-BC *No.* (%)
29c	Urine	Up-regulation	6 (50.0%)	3 (100.0%)	13 (68.4%)	9 (69.2%)	1(12.5%)
		Down-regulation	6 (50.0%)	0	6 (31.6%)	4 (30.8%)	7 (87.5%)
		total	12	3	19	13	8
	Tissue	Up-regulation	3 (100.0%)	4 (100.0%)	15 (62.5%)	7 (58.3%)	4 (66.7%)
		Down-regulation	0	0	9 (37.5%)	5 (41.7)	2 (33.3%)
		total	3	4	24	12	6
	P		0.229 F NS	-----	0.686 C NS	0.688 F NS	0.091 F NS
125	Urine	Up-regulation	7 (63.6%)	3 (100.0%)	8 (40.0%)	11 (84.4)	4 (50.0%)
		Down-regulation	4(36.4%)	0	12 (60.0%)	2 (15.6)	4(50.0%)
		total	11	3	20	13	8
	Tissue	Up-regulation	1 (33.7%)	4 (100.0%)	17 (68.0%)	8 (66.7%)	1 (20.0%)
		Down-regulation	2 (66.7%)	0	8 (32.05)	4 (33.3%)	4 (80.0%)
		total	3	4	25	12	5
	P value		0.538 C NS	----	0.060C NS	0.015 F S	0.565 F NS
141	Urine	Up-regulation	6 (50.0%)	0	7 (35.0%)	5 (41.7%0	2 (25.0%)
		Down-regulation	6 (50.0%)	3 (100.0%)	13 (65.0%)	7 (58.3%)	6 (75.0%)
		total	12	3	20	12	8
	Tissue	Up-regulation	0	2 (50.0%)	11 (44.0%)	4 (33.3%)	3 (60.0%)
		Down-regulation	3 (100.0%)	2 (50.0%)	14 (56.0%)	8 (66.7%)	2 (40.0%)
		total	3	4	25	12	5
	P value		0.235 F NS	0.429 F NS	0.540 C NS	1.000 F NS	0.293 F NS
145	Urine	Up-regulation	7 (58.3%)	2 (66.7%)	8 (61.9%)	9 (61.9%)	3(37.5%)
		Down-regulation	5 (41.7%)	1(33.3%)	13 (38.1%)	4(38.1%)	5 (62.5%)
		total	12	3	21	13	8
	Tissue	Up-regulation	1 (33.3%)	4 (100.0%)	18 (69.2%)	6 (50.0%)	2 (40.0%)
		Down-regulation	2 (66.7%)	0	8 (30.8%)	6 (50.0%)	3 (60.0%)
		total	3	4	26	12	5
	P value		0.569 F NS	0.429 F NS	0.033 C NS	0.428 F NS	1.000 F NS
205	Urine	Up-regulation	9 (75.0%)	0	10 (52.6%)	3 (23.1%)	2 (25.0%)
		Down-regulation	3 (25.0%)	3(100.0%)	9 (47.4%)	10 (76.9%)	6 (75.0%)

Tissue	total	12	3	19	13	8
	Up-regulation	1 (33.3%)	4 (100.0%)	15 (62.5%)	5 (41.7%)	2 (40.0%)
	Down-regulation	2 (66.7%)	0	9 (37.5%)	7 (58.3%)	3 (60.0%)
	total	3	4	24	12	5
P value		0.242 F NS	0.029 F S	0.515 C NS	0.111 F NS	1.000 F NS

No: represent the number, F: Fischer exact test; S: Significant; NS: Not significant at $P > 0.05$

4. Discussion

Bladder cancer is a complex disease with different molecular and pathological pathways, reflecting different behaviours depending on the clinical staging of the tumor and molecular type [16]. In this study, 49/50 patients were diagnosed histopathologically as having TCC-BC and 1/50 patient had adenocarcinoma bladder cancer. The ratio of male to female was 3.4:1. This percentage was reported due to numerous potential biologic and epidemiologic factors that probably underlie the gender differences observed in bladder cancer incidence, stage at time of diagnosis and outcomes such as smoking status, potential molecular mechanisms include disparate metabolism of carcinogens by hepatic enzymes between men and women, resulting in differential exposure of the urothelium to carcinogens, as well as activity of the sex steroid hormone pathway may have played a role in bladder cancer development [17]. An Iraqi study from Urology Clinic of Baghdad Teaching Hospital in 2019 included 66 patients with TCC with the age ranging 24–90 years. Male percentage was 89.4% 59 while for females it was 10.6% [18].

One of more important risk factors is age About 9 out of 10 people with bladder cancer are older than 55 and cases before the age of 40 are rare and are usually benign in nature [19, 20]. In this study, patients with low-grade tumors (Ta, T1) were 25/39 (65.8%) while muscle-invasive cancers (MIBC), also known as high-grade tumors (T2, T3 and T4), were found in 14/39 (35.2%) patients. A retrospective study in Baghdad in 2018 included 32 cases of TCC bladder cancer. 18/32 (56.25%) cases had papillary high-grade urothelial carcinoma, but 7/32 (21.88%) cases had low-grade papillary [21]. A retrospective Jordanian study in Amman included 249 patients with TCC. 212 (85.1%) males and 37 (14.9%) females in period extending from 2008-2017, out of them, 128 (51.4%) patients were between the age range of 65-84 years. Among all cases143 (59.3%) patients had low grade, while 98 (40.7%) patients had high grade [22]. A study in Saudi Arabia in 2019 included 66 patients with TCC/BC (male to female ratio was (10:1) (60 to 6), aged between 39-94 years) and 78 healthy control of those, 45(68.2%) patients with high grade and 21 (32%) patients with low tumor grade [23]. Bladder cancer is usually of non-muscle-invasive cancers (NMIBC) type and is detected early at the time of initial diagnosis. Studies referred to that 75-85% of all BCs are non-muscle invasive with only 20-40% of them progressed to invasive type within 5 years of primary treatment which is due to the fact that urothelial malignancies are an ongoing phenomenon, and patient with BC needs a more regular and systematic follow-up with appropriate adjuvant therapy [24].

In the current study 10/49 (20%) patients had TCC/BC recurrence which ranged between 8 to 10 months. Globally, recurrence rate following the transurethral resection of bladder tumors (TURBT) can be up to 75% within 5 years [25]. A Turkish study done in 2017 referred to that 41/81 (50.6%) of patients with TCC/BC showed recurrence [26]. In this study results showed that selected miRNAs (miR-29c, miR-125, miR-141, miR-145 and miR-205) were differentially expressed in patients negative to TCC/BC and patients with different stages of TCC/BC (in tissue biopsies and urine samples). Abnormalities in the miRNA biogenesis

pathway may affect the miRNA expression profile and influence the progression of numerous human body system cancers, including urinary system cancers [27]. In the current study, fold change in expression level of studied miRNAs according to groups varied which could be due to the differences in number of samples collected from each included subjects groups (patients with TCC-BC, patients with adenocarcinoma and patients with prostate cancer). In addition, there were no significant differences between urine and tissue biopsies samples. Changes in expression level of miRNA may act as a good predisposing biomarker for bladder cancer since aberrant expression profiles of miRNAs are widely noticed in the clinical tissue specimens and urine samples, in addition to blood samples from patients with bladder cancer which could closely represent the pathological features of bladder cancer, such as the tumor stage/grade, metastasis, recurrence and chemo-sensitivity [28].

Up-regulation of miR-29c from patients with TCC/BC, in both urine and tissue biopsies, was 60.9% and 62.7% respectively. MiR-29c mediates the proliferation and apoptosis of bladder cancer by regulating the expression of members of the Bcl-2 family genes [29]. A Japanese study identified a panel of miRNAs that could be used to predict aggressive phenotype from normal nonaggressive of BC by estimating expression levels of these miRNAs using real-time quantitative PCR technique and to estimate the prognostic value of nine specific miRNAs markers in 84 patients with TCC. They found dysregulation of 9 miRNAs, including (hsa-miR-29c, hsa-miR-125b-5p, hsa-miR-145-5p) miR125b that were associated with high risk while miR29c and miR145 were shown to be protective [30]. In the current study, change in expression level of miR-125 in patients with TCC/BC was statistically highly significant between non-muscles invasion and muscles invasion bladder cancer. Hence, miR-125 could act as prognosis biomarker in the early stage of TCC-BC. The function of miR-125b diverges in different cancers depending on the different molecular contexts and the tumor microenvironment such as down regulating in bladder cancer is promoted by reducing the inhibition of genes that promote proliferation, differentiation and apoptosis inhibition [31]. A German study in 2013 included 40 patients of which 25 had NMIBC, 15 had MIBC and 17 subjects were used as control. After screening for 723miRNAs using real-time quantitative PCR, they found that 7 miRNAs (including miR-141 and miR-205) were up-regulated and eight miRNAs (including miR-125b and miR-145) were down-regulated in bladder tissue samples compared to healthy tissue control [32]. In the current study, TCC/BC patients, expression level of miR-141 varied in urine and tissue samples, but generally down-regulation of expression level was seen in both samples. This is referred to that miR-141 may be regulation of this miRNA was the same between urine and tissue biopsies in TCC/BC patients. MiR-141 related with tumorigenesis, invasion and metastasis through targeting PTEN gene (Phosphatase and Tensin Homolog) which is responsible for signalling in cell cycle [33]. In the current study fold change in expression level of miR-145 in urine samples from patients with TCC/BC was up-regulation in (51.2%) of urine samples, while down-regulation in (49.8%) of samples. In the same group of patients, up-regulation in expression level of miR-145 in tissue biopsies samples was seen in 62.7% of samples, while down-regulation was seen in 37.3% of samples. MiR-145 is considered vital factor for tumor aggressiveness and prognosis which may serve as candidate biomarker for diagnostic and prognostic purposes through targeting PTEN signalling pathways. Also expression level of miR-145 has been associated with epithelial mesenchymal transition (EMT) score [33]. A study in Romania in 2020 included patients with TCC to compare expression level of 7 microRNAs (including miR- 145-3p and miR-145-5p) from cancer tissue and from normal tissue using Taq man Real-Time quantitative PCR. Their results indicated a significant down-regulation of miR-145-5p and miR-145-3p in cancer tissue, compared to normal tissues [34].

In the current study, fold change in expression level of miR-205 was up-regulation in 53.6% of urine samples from patients with different stages of TCC/BC, while down-regulation was seen in 46.4%)patients. Up-regulation in gene expression level of miR-205 was seen in 55.8% of tissue biopsies samples from patients with different stages of TCC-BC, while down-regulation was seen in 44.2% of tissue biopsies samples. MiR-205 is related with tumorigenesis invasion and metastasis. Though target in zeb1, zeb2 genes (zinc-finger transcription factor) is responsible for adherent junctions, focal adhesion also participates in the epithelial-mesenchymal transition (EMT), thus enhancing the invasiveness and metastatic ability of bladder cancer cells [33].

5. Conclusion

miR-125 could be associated with the development of invasive stages of TCC/BC.

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7. Data Availability

The datasets used or/and analysed during the current study was available from the corresponding author on reasonable request.

8. Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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