RESEARCH ARTICLE

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Relapse and Survival in Bladder Cancer Patients Undergoing microRNA-129 and microRNA-145 Assays

Mostafa M M ELfieky[1], Mohamed Abd El Rahman[2,3]*, Aysam M Fayed[1,4], Zahraa Haleem Al-Qaim[5], Ahmed Khalid Aldhalmi[2], Eman AE Badr[6], Amal Abdel Aziz[1], Gehan M A Ibrahim[1]

Abstract

Objective: The lack of indicators to measure tumor's invasive biological behavior is an important issue. The aim of this study was to examine the effect of miRNAs 129 and 145 on tumor progression as well as patient survival. **Method:** Seventy five breast cancer (BC) patients and 75 controls were included in this research. Two miRNA expressions were estimated using real-time PCR. Biomarkers for BC detection was tested using ROC curves and AUC. **Result:** *miR-129* and *miR-145* expressions were significant. *miR-129* and miR-145 classifiers (AUC = 0.943 and 0.748, respectively) help diagnose BC. Unlike *miR-145*, *miR-129* did not affect the Kaplan–Meier survival curve analysis for progression-free survival at the end of the trial. The development of transitional cell carcinoma disease was found to have a strong correlation with *miR-145* in both univariate and multivariate Cox regression analyses. Additionally, infiltrating + invasive urothelial carcinoma was also found to be correlated with miR-145. Conversely, elevated *miR-129* expression in BC patients did not lead to an increase in cancer-specific recurrence or mortality, as observed in both univariate and multivariate Cox regression studies. **Conclusion:** The miRNA signature can help detect survival-associated miRNAs and develop BC miRNA therapeutics.

Keywords: Bladder cancer- microRNA 129- microRNA 145- relapse- survival

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Introduction

Bladder cancer (BC) ranks 7th and 14th in men and women worldwide, respectively [1]. In industrialized countries, men develop BC. It remains the most frequent urinary cancer [2]. Most BCs (stages Ta and T1) are nonmuscle invasive and successful. T2–T4 muscle-invasive bladder tumors are distantly invasive [3,4]. BC is primarily hereditary and environmental [5]. Despite advancements in surgery, radiation, and chemotherapy, breast cancer remains highly fatal [6]. Established BC biomarkers have many diagnostic limitations [7-10]. Novel markers are required to identify and predict BC at an early stage.

Posttranscriptionally, microRNAs (miRNAs) influence gene expression and become tumor suppressors or oncogenes [11,12]. BC miRNA dysregulation was associated with BC [13-15]. Thus, miRNAs can detect cancer. Prior studies demonstrated that miRNAs alter BC cell motility, invasion, proliferation, and survival.

Several studies reported *miR-129*-5p to be the most downregulated in spinal tissues. *miR-129*-5p overexpression improved spinal tissue inflammation, apoptosis, and functional recovery in injured animals [16,17]. Tumor growth and progression involve miR-145-5p. Few studies explored BC and miR-145-5p. miR-145-5p was found in BC cell proliferation and migration [18,19]. The aim of this study was to examine the effect of miRNAs 129 and 145 on tumor progression as well as patient survival.

Materials and Methods

In this study, 150 participants were included: 75 with BC and 75 healthy volunteers confirmed using cystoscopy. This research was conducted at the Clinical Urology Department, Faculty of Medicine, Menoufia University, from November 2021 to December 2022.

*[1]Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt. [2]College of Pharmacy, Al-Mustaqbal University, Babylon, 51001, Iraq. [3]Clinical Pharmacy Department, Badr University Hospital, Faculty of Medicine, Helwan University, Egypt. [4]Medical Laboratory Techniques Department, College of Health and Medical Technique, Al-Mustaqbal University, Babylon,51001, Iraq. [5]Department of Anesthesia Techniques, Al-Mustaqbal University College, Iraq. [6]Medical Biochemistry and Molecular Biology, Faculty of Medicine, Menoufia University, Egypt. *For Correspondence: mohamedmahmoud@mustaqbal-college.edu.iq*

Menoufia University Faculty of Medicine Medical Research Ethics Committee approved the experiment. Radiology, cystoscopy, histology, and clinical tests confirm the diagnosis of BC. Cancer was staged and graded using TNM and WHO standards. Patients with BC had isolated lesions on ultrasound and CT imaging. Sex, age, domicile, socioeconomic level, pathological subtype, grade, smoking, hematuria, relapse or progression use status, and other confounding risk markers were collected in structured personal interviews. All patients consented, knowing the risks.

In total, 3 mL of blood was collected from each participant via aseptic venipuncture. Sample division: 2 mL of whole blood was put into a simple sterile tube and centrifuged at 3500 rpm for 15 min. The serum was used to measure creatinine, urea, and Hb. RT-qPCR RNA Extraction. RNeasy Mini Kit and Qiazol Reagent (Qiagen, Germany) were used to extract total RNA containing miRNA to quantify *miR-145* and *miR-129* in BC tissues. RNA purity was confirmed using a NanoDrop spectrophotometer (Thermo Scientific, USA). Per instructions, the miScript II First Strand cDNA kit (Qiagen, Germany) reverse-transcribed RNA. The reactions were incubated at 25˚C for 5 min, 42˚C for 60 min, and 70˚C for 5 min in a 2720 Applied Biosystems thermal cycler. RT-qPCR was performed using Singapore SYBR Green PCR Master Mix (Life Technologies, USA) and a thermal Real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Inc.). After a 10 min incubation at 94˚C, the reactions went through 45 cycles of 15 s at 95˚C and 30 s at 60˚C. Primers were designed as follows:

miR-145- 5p	Forward	5′-CCTTGTCCTCACGGTCCAGT-3′
	Reverse	5′-AACCATGACCTCAAGAACAGTATTT-3′
miR-129-5p	Forward	'- CTTTTTGCGGTCTGGGCTTGC -3′
	Reverse	5′- AGCAAGCCCAGACCGCAAAAA -3′
U6	Forward	5′-CTCGCTTCGGCAGCACA-3′
	Reverse	5′-AACGCTTCACGAATTTGCGT-3′
18SrRNA	Forward	5′-GCGGTTCTATTTTGTTGGTTT-3′
	Reverse	5′-ATCGCCGGTCGGCATCGTTT -3′

U6 controlled *miR-145* and miR129. The 2_DDCt technique (Livak and Schmittgen, 2001) evaluated miR-145-5p and miR129-5p levels on an Applied Biosystems 7500 Real-Time PCR System (Foster City, CA, USA).

Statistical Analysis

Computer data were analyzed using IBM SPSS 20.0. A percentage and number represented quantitative data. Only BC deaths were examined. At the last follow-up, censored patients did not attain the endpoint. The survival curves were generated using the Kaplan–Meier method. Survival distribution tests were conducted on subgroups using the log-rank method. Multivariate Cox proportional hazards regression analysis examined miRNA levels' predictive power after controlling for other factors. Significant differences between populations were observed at confidence levels above 95% ($p < 0.05$) in all two-sided tests. The survival study used Fisher's Exact or Monte Carlo correction, Student's t-test, Mann–Whitney test, and Chi-square test. Data significance was at the level of 5%.

Results

The participants included 75 BC patients (mean: 68.93 ± 8.31 years), with 51 males and 24 females. Of 75 healthy volunteers, 47 males and 28 females were free of chronic diseases and had a mean age of 67.19 ± 6.14 years. Moreover, 75 BC cases were grouped by age (45–73 years) and smoking (25 negative and 50 positive). BC patients and controls shared gender, age, and smoking rates.

Clinicopathological characteristics of the patient's population

The clinical characteristics of BC are listed in Table 2. Pathological subtypes were transitional cell carcinoma (89.3%), urothelial carcinoma (6.7%), and infiltrating + invasive carcinoma (4.0%). The grades found were as follows: Grades I (22.7%), II (41.3%), and III (36.0%) appear. Hematuria indicated a 40% tumor size compared to that revealed by the CT (60%, ≤3 vs. 40%, >3). Prevalence of no recurrence (85.3%) was more significant than that of advancement (14.7%) and duration per month (mean: 11.41 ± 1.53) (Table 1).

Significant variations in urea, creatinine, and Hb levels were found between patients and controls ($p \leq 0.001$). Table 2 shows that the case group had significantly lower *miR-129* and *miR-145* expressions than the control group ($p = 0.001$).

Correlation between the miR-129 and miR-145 expressions with personal, clinicopathological, and biochemical parameters

Hb, pathological subtype, and grade were positively linked with *miR-129* ($r = 0.320$, $p = 0.005$). In the cases group, miRNA 145 was strongly correlated with CT tumor size and Hb ($r = 0.241$, $p = 0.037$; $r = 0.306$, $p = 0.008$). Higher grade stage and CT tumor size decreased *miR-129* and *miR-145* expressions ($p = 0.003$ and 0.008, respectively; table not supplied).

miR-129 and miR-145 bladder cancer diagnostic effectiveness

ROC curve analysis revealed that *miR-129* and *miR-145* differentiated patients from controls. The ROC curve analysis for *miR-129* showed an AUC of 0.943 (95% CI = 0.907–0.98; $p < 0.001$). The values were as follows: diagnostic sensitivity of 94.67%, specificity of 85.5%, PPV of 85.5%, and NPV of 94.0% with maximum at a threshold of ≤1.287 ($p < 0.001$). ROC curve analysis for *miR-145* showed an AUC of 0.748 (95% CI = 0.665–0.831; $p < 0.001$), with a diagnostic sensitivity of 88.0%, specificity of 62.67%, PPV of 70.2%, and NPV of 83.9%, peaking at ≤0.987 ($p < 0.001$), as shown in Figure 1.

Progression-free survival data of the cases

Research indicated no significant difference in progression-free survival between patients with low and high miRNA 129 expression ($p > 0.246$; mean = 11.250, 77.5%; mean = 11.429, 88.6%). Concurrently, there was a significant difference in progression-free survival between

Table 1. Clinical-Pathological Features of Patients

	No.	%
Pathological subtype		
Transitional cell carcinoma	67	89.3
Urothelial carcinoma	5	6.7
Infiltrating + Invasive urothelial carcinoma	3	4
Grade		
Grade I	17	22.7
Grade II	31	41.3
Grade III	27	36
CT tumor size		
≤3	45	60
>3	30	40
Min. – Max.	1.50 – 7.50	
Mean ± S.D.	3.20 ± 1.31	
Median (IQR)	3.0 (2.05 – 3.95)	
Hematuria		
Negative	45	60
Positive	30	40
Relapse or progression status		
No relapse	64	85.3
pt. relapsed	11	14.7
Duration (months)		
Min. – Max.	6.0 – 12.0	
Mean ± S.D.	11.41 ± 1.53	
Median (IQR)	12.0 (12.0 – 12.0)	

patients with low and high miRNA 145 expressions at the end of the study (p < 0.001; mean = 10.718, 66.7%; mean = 12.0, 100%), as shown in Figure 2.

The relationship between bladder cancer patients' clinicopathological traits and relapse

Univariate Cox regression analysis showed that parametric data were nonsignificant as disease progression predictors: age (p = 0.375), sex (female; p = 0.284), smoking (p = 0.781), tumor size (p = 0.772), hematuria (p = 0.735), urea (p = 0.801), creatinine (p = 0.864), and Hb (%) (p = 0.914). Regarding the pathological subtypes, transitional cell carcinoma and infiltrating + invasive urothelial carcinoma were significant predictors of disease progression (p = 0.007 and p = 0.030, respectively), whereas urothelial carcinoma and Grade III were not significant predictors of disease progression (p = 0.030 and p = 0.121, respectively). As for miR-145, it was an important predictor of disease progression (p < 0.001), whereas *miR-129* was not (p = 0.211). Transitional cell carcinoma (p = 0.007) and *miR-145* were significant disease progression predictors in multivariate Cox regression analysis (p<0.001), whereas infiltrating + invasive urothelial carcinoma was not a significant predictor (p = 0.357). Concurrently, all other parameters were nonsignificant (Table 3).

The relationship between bladder cancer patients' clinicopathological traits and mortality

Univariate Cox regression analysis for case group mortality parameters showed that parametric data were not associated with disease progression: age (p = 0.409), sex (female) (p = 0.268), smoking (p = 0.806), tumor size (P=0.837), hematuria (p = 0.687), urea (p = 0.897), creatinine (p = 0.870), and Hb (%) (p = 0.939). Transitional cell carcinoma (p = 0.014) and *miR-145* were associated with disease progression (p < 0.001) in univariate Cox regression analysis for death in the cases group.

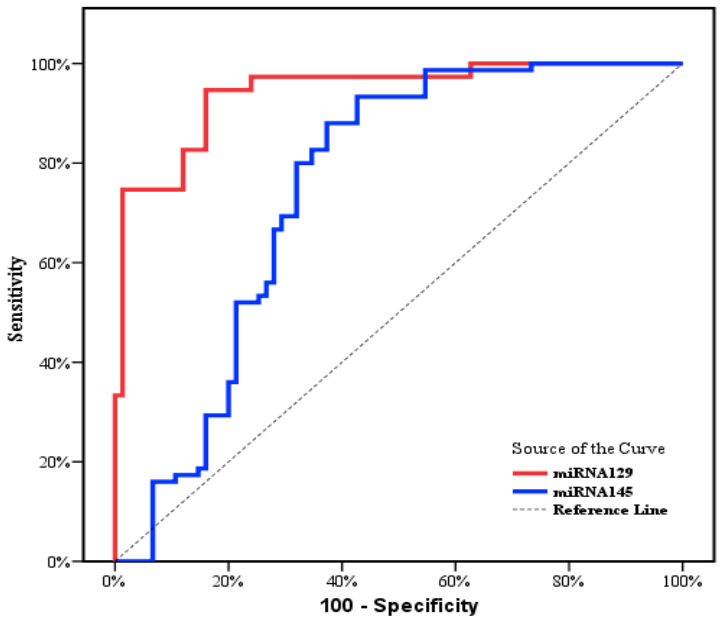

Figure 1. ROC Curve for miRNA 145 and miRNA 129 to Discriminate Patients (n = 75) from Control (n = 75). ROC curve analysis found that miR-129 and miR-145 discriminated patients from control. The ROC curve analysis for miR-129 showed an AUC of 0.943 (95% CI=0.907-0.98, P <0.001). Diagnostic sensitivity (94.67%), specificity (85.5%), PPV (85.5%), and NPV (94.0%) maximum at a threshold of ≤1.287 (P<0.001). ROC curve analysis for miR-145 showed an AUC of 0.748 (95% CI=0.665-0.831, P <0.001), with diagnostic sensitivity (88.0%), specificity (62.67%), PPV (70.2%), and NPV (83.9%) peaking at ≤0.987 (P<0.001).

Table 2. Lab Tests and miRNA Levels in the Two Groups

	Patients (n = 75)	Control (n = 75)	t	p
Urea (mg/dl)				
Min. – Max.	47.50 – 69.0	22.0 – 34.0		
Mean ± S.D.	53.36 ± 4.27	27.20 ± 3.07	43.099*	<0.001*
Median (IQR)	52.0 (50.40 – 56.45)	28.0 (25.0 – 30.0)		
Creatinine (mg/dl)				
Min. – Max.	1.60 – 2.40	0.80 – 1.10		
Mean ± S.D.	1.97 ± 0.19	0.96 ± 0.09	41.950*	<0.001*
Median (IQR)	1.90 (1.80 – 2.10)	0.97 (0.90 – 1.0)		
Hb (gm/dl)				
Min. – Max.	8.90 – 12.0	9.40 – 13.20		
Mean ± S.D.	10.49 ± 0.80	11.99 ± 1.03	9.999*	<0.001*
Median (IQR)	10.50 (9.85 – 11.0)	12.0 (11.60– 12.85)		
MiRNA129-5p				
Min. – Max.	0.44 – 2.23	0.76 – 2.80		
Mean ± S.D.	0.88 ± 0.33	1.91 ± 0.62	323.0*	<0.001*
Median (IQR)	0.85 (0.66 – 0.95)	1.75 (1.40 – 2.60)		
MiRNA145-5p				
Min. – Max.	0.22 – 2.23	0.08 – 3.83		
Mean ± S.D.	0.66 ± 0.37	1.54 ± 1.07	1416.0*	<0.001*
Median (IQR)	0.55 (0.46 – 0.78)	1.37 (0.59 – 2.30)		

IQR, Interquartile range; SD, Standard deviation; t, Student's t-test; p, p value for comparing the two studied groups; *, Statistically significant at $p \leq 0.05$

Urothelial carcinoma was not significant (p = 0.102), nor was infiltrating + invasive urothelial carcinoma (p = 0.069), Grade (III) (p = 0.151), or miRNA 129 (p = 0.168). Transitional cell carcinoma (p = 0.004) and *miR-145* (p < 0.001) were significant predictors of disease progression in multivariate Cox regression analysis for case mortality, whereas all the other parameters were nonsignificant (Table 4).

Discussion

According to the latest data from the Egyptian National Cancer Institute registry, the prevalence of BC is 10.1% [20]. BC, which is the sixth most common cancer in the

Table 3. Cox Regression Univariate and Multivariate for Case Group Relapse Parameters

	Univariate		#Multivariate	
	P	H.R. (L.L. – U.L. 95%C.I)	p	H.R. (L.L. – U.L. 95%C.I)
Age (years)	0.375	1.033(0.961 – 1.111)		
Sex (female)	0.284	1.815(0.610 – 5.402)		
Smoking	0.781	1.182(0.364 – 3.839)		
Pathological subtype				
Transitional cell carcinoma	0.007*	0.198(0.061 – 0.647)	0.036*	0.160(0.029 – 0.888)
Urothelial carcinoma	0.098	3.577(0.789 – 16.207)		
Infiltrating + Invasive urothelial carcinoma	0.030*	5.404(1.179 – 24.764)	0.357	2.682(0.328 – 21.929)
Grade (III)	0.121	2.371(0.796 – 7.065)		
C.T. tumor size	0.772	1.061(0.710 – 1.587)		
Hematuria	0.735	1.208(0.406 – 3.594)		
Urea	0.801	1.016(0.898 – 1.149)		
Creatinine	0.864	0.775(0.043 – 14.109)		
H.B. (%)	0.914	1.038(0.525 – 2.056)		
miRNA 129	0.211	0.237(0.025 – 2.265)		
miRNA 145	0.001*	0.005(0.0 – 0.113)	0.001*	0.001(0.0 – 0.057)

HR, Hazard ratio; CI, Confidence interval; LL, Lower limit; UL, Upper limit; #, All variables with p < 0.05 were included in the multivariate; *, Statistically significant at $p \leq 0.05$.

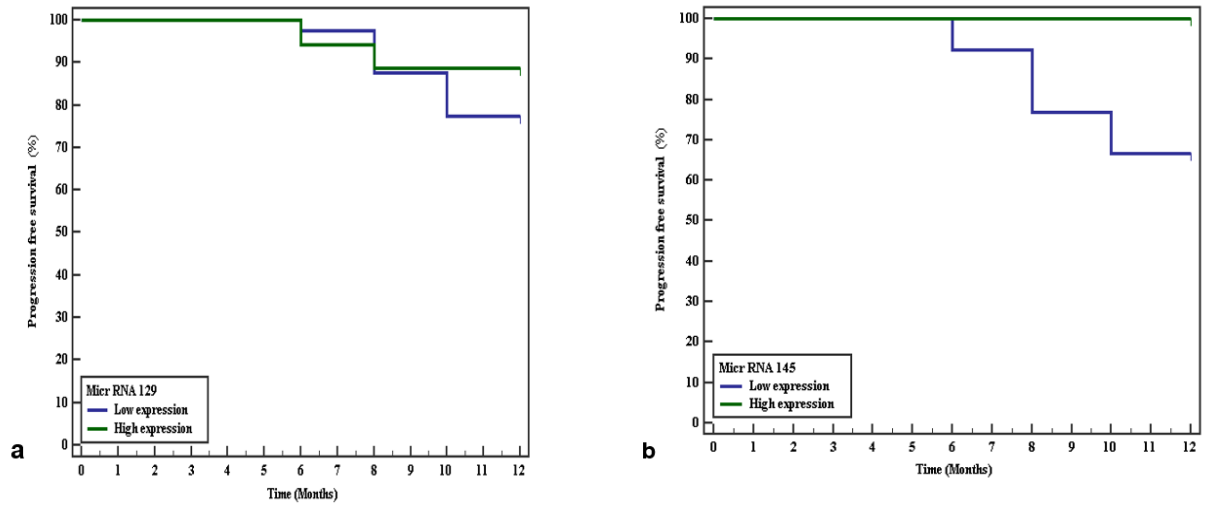

Figure 2. Kaplan-Meier Survival Curve for Progression-Free Survival with MiRNA 129 and 145 a and b. No significant difference in progression-free survival between patients with low and high micro-RNA 129 expression was found at the end of the study (P > 0.246, Mean= 11.250, 77.5% & 11.429, 88.6%), while there was a significant difference in progression-free survival between patients with low and high micro-RNA 145 expression at the end of the study (P<0.001, mean 10.718, 66.7% & 12.0, 100%).

Table 4. Univariate and Multivariate Cox Regression Analysis for Case Group Mortality Parameters

	Univariate		#Multivariate	
	P	H.R. (L.L. – U.L. 95%C.I)	P	H.R. (L.L. – U.L. 95%C.I)
Age (years)	0.409	1.031(0.959 – 1.108)		
Sex (female)	0.268	1.851(0.622 – 5.510)		
Smoking	0.806	1.159(0.357 – 3.764)		
Pathological subtype				
Transitional cell .carcinoma	0.014*	0.228(0.070 – 0.742)	0.004*	0.142(0.038 – 0.533)
Urothelial carcinoma	0.102	3.530(0.779 – 15.992)		
Infiltrating + Invasive urothelial carcinoma	0.069	4.045(0.896 – 18.254)		
Grade (III)	0.151	2.225(0.748 – 6.623)		
C.T. tumor size	0.837	1.043(0.696 – 1.563)		
Hematuria	0.687	1.251(0.420 – 3.727)		
Urea	0.897	1.008(0.892 – 1.140)		
Creatinine	0.87	0.784(0.043 – 14.340)		
H.B. (%)	0.939	1.027(0.516 – 2.043)		
miRNA 129	0.168	0.199(0.020 – 1.971)		
miRNA 145	<0.001*	0.003(0.0 – 0.083)	<0.001*	0.142(0.038 – 0.533)

HR, Hazard ratio; CI, Confidence interval; LL, Lower limit; UL, Upper Limit; #, All variables with p < 0.05 were included in the multivariate; *, Statistically significant at p ≤ 0.05

United States, is uncommon among individuals under the age of 40 [21]. Complexity and limited therapeutic efficacy make BC, the most common urinary system cancer, worse [22]. Thus, BC mechanisms and new treatments must be understood. Ta, T1, and T2-4 are muscle-invasive cancers, and malignancies exist. Long-term follow-up shows that benign, noninvasive papillary tumors seldom become muscle-invasive, up to 60% [23]. Many high-throughput studies examined genetic alterations and gene expression in BC progression [3].

miRNAs regulate mammalian gene expression and physiology [24,25]. Numerous studies demonstrated that improperly produced miRNAs disrupt well-controlled cellular RNA networks, promoting cancer cell growth, progression, and metastasis. Cancer cells' aberrant miRNAs and RNA network modifications explain growth and metastasis. BC cell growth requires dysregulated miRNAs [14]. In the above scenario, we examined *miR-129* and *miR-145* expression levels as diagnostic, prognostic, and therapeutic biomarkers.

UBC is predominantly male, according to the textbook [26]. Smoking and cancer-causing substances may increase in men [27]. Pakistan [28], Netherlands [29], Malaysia [30], and Nigeria [31] investigations support this. UBC instances were 5:1 M: F; however, Rambau et al. [32] from Tanzania showed a female prevalence.

Men accounted for 68% of bladder cancer cases in 2019 [33]. Again, Lavery et al. [34] discovered 102 men and 27 females in 129 instances.

Transitional cell carcinoma (TCC) was the most prevalent histological pattern (89.3%), followed by urothelial carcinoma (6.7%) and infiltrating + invasive (4.0%). Similar high TCC rates also exist elsewhere. TCC is the most prevalent BC, according to textbooks and WHO data [26]. However, rural Africans may have lower bladder TCC rates due to lesser chemical exposure [35]. Nepalese Vaidya [36] reported 97.6% TCC. Mubarak et al. [28] found 94.3% TCC in Pakistan. In 2010, the Netherlands cancer registry reported over 90% of TCC urothelial tumors [29]. Kong et al. [30] found 90.4% TCC in Malaysia. According to El-Siddig et al. [37], 70% of TCC cases were superficial and 30% muscle-invasive.

According to Cohen and Brown [38], 40% hematuria was found. Rafique and Javed [39] found 78.6% hematuria, while Ragab et al. [40] reported 72.5%. Gupta et al. [41] found painless hematuria in 40% of BC patients. The majority of BC patients who undergo cystoscopy and provide sufficient urine samples have microhematuria. The tumors were histologically classified using the WHO (1999)/ISUP urothelial neoplasm grading system [42]. First-line testing includes cystoscopy and urine cytology. Cystoscopy is the most effective method for staging and diagnosing BC. Cancers can be classified into high-grade and low-grade categories [43]. Based on aggressiveness, low-grade cancer cells grow slowly, seem normal, and operate like healthy cells, whereas high-grade cells expand quickly, look disordered, and are more likely to go into the bladder muscle layer.

In a study conducted in Nepal, it was shown that 52.2% (n = 120) of the masses were classified as high-grade. Out of these masses, 77.3% were categorized as II (31 cases, 41.3%), III (27 cases, 36.0%), and I (17 cases, 22.7%). However, El-Siddig et al. [37] discovered that two-thirds of the cases were low-grade and one-third were high-grade. Researchers from Malaysia documented that 32.5% of cases of high-grade superficial TCC were observed [30]. In Southern Pakistan, research found a ratio of 3/4 low to ¼ excellent quality [28]. Low-grade papillary urothelial carcinoma was 53.85% more common than high-grade (34.61%), according to Laishram et al. [44,45], with percentages of 44% and 29.5%, respectively. In Amman city, Al Khader et al. [46] found 57 (58.2%) and 5 (5.1%) high-grade cancers in 65–84-year-old and >84-year-old individuals.

CT helps in determining the localization of tumors [47]. CT displays BC diagnostics, tumor stage, and therapeutic selection vascular structure. Liu et al. [48] reported tumor CT size ranging from ≤3 cm for 60% to >3 cm for 40%. CT accuracy depends on bladder tumor lesion size. Many studies revealed that microRNAs (miRNAs/miRs) promote oncological and nononcological illnesses through biological signaling networks. miRNAs are now of interest to BC researchers [49]. Small, noncoding RNAs impede translation, lowering target gene expression. In this study, we investigated the effects of *miR-129* on gene expression [50]. The study found that BC patients had less *miR-129* and *miR-145* expression than those in controls. Volinia et al. observed that miR-143 (2.6-fold) and *miR-145* (7.0-fold) were most downregulated. Most *miR-145* was found in lymphocytes and connective tissue. *miR-145* and miR-143 were downregulated in various malignancies and scarcely expressed in carcinoma and normal urothelial cells [51,52]. The *miR-129*-upregulated genes in advancing samples were target site-rich. TP53INP1 and *miR-129* targets are involved in melanoma [53]. *miR-145* and *miR-129* predict BC prognosis [54,55].

Prostate cancer tissues have less *miR-129*-5p, according to Gao et al. [56]. In BC, *miR-129* had the largest overexpression compared to those in previous studies [55,57]. Prior work indicated that *miR-129*-5p overexpression decreases ZIC2 expression, impacting the Wnt/β-catenin pathway. E-cadherin expression increased, whereas phosphorylated Wnt, β-catenin, N-cadherin, and vimentin decreased. By targeting ZIC2, *miR-129*-5p may diminish EMT, angiogenesis, tumorigenesis, and migration. Our findings complemented those of Zabolotneva et al. [57], who found that *miR-145* and miR-133a were downregulated in cancer tissues and could distinguish cancer cells from noncancer cells with >70% sensitivity and >75% specificity.

Hb, pathogenic subtype, and grade positively correlated with *miR-129* ($r = 0.320$; $p = 0.005$). Low *miR-129* expression in peripheral blood mononuclear cells was linked to aggressive clinical-pathological features like histological grade ($p = 0.010$), high preoperative PSA level ($p = 0.002$), pathological stage ($p = 0.011$), high Gleason score ($p = 0.005$), lymph node metastasis, angiolymphatic invasion, and biochemical recurrence [58]. Hb, sex, and CT tumor size were strongly correlated with miRNA 145 ($r = 0.241$, $p = 0.037$; $r = 0.306$, $p = 0.008$). Researchers identified a favorable connection between the high tumor stage and low miR-145. Other studies discovered that linked *miR-145* underexpression to cancer and high-grade urothelial carcinomas downregulated *miR-145* but not low-grade ones [59,60].

Ichimi et al. [13] detected *miR-145* underexpression in BC using microarray and qRT-PCR. They did not link *miR-145* expression to histological grading, staging, or tumor behavior. Dip et al. [61] showed no effect of *miR-145* expression on histological grade, tumor stage, angiolymphatic neoplastic invasion, or recurrence. Research revealed that miRNA 129 may distinguish patients from controls ($p < 0.001$), with an AUC of 0.943 (95% CI: 0.907–0.978) at cutoff ≤1.287, resulting in 94.67% sensitivity, 84.0% specificity, and 85.5% PPV.

MicroRNA 145 can distinguish patients from controls ($p < 0.001$), with an AUC of 0.748 (95% CI: 0.665–0.831), at cutoff ≤0.987, achieving 88% sensitivity, 62.67% specificity, and 70.2% PPV. However, low and high levels of miRNAs 129 and 145 did not affect survival time (mean = 11.500, 87.5%; mean = 11.590, 89.7%) or high levels (mean 11.314, 82.9%; mean = 11.222, 80.6%). The multivariate study showed that prostate cancer patients severely downregulated *miR-129*, a novel independent prognostic factor [58,62]. The BC miRNA profile shows numerous down- and upregulated miRNAs [63-66]. miRNAs may diagnose, prognose, and treat BC [64,65]. Significant indicators for disease progression in univariate

and multivariate Cox regression analysis include TCC ($p = 0.007$), infiltrating + invasive urothelial carcinoma ($p = 0.030$), and *miR-145* ($p < 0.001$). In Table 4, univariate and multivariate Cox regression analysis revealed TCC ($p = 0.014$; $p = 0.004$) and *miR-145* as significant disease development predictors ($p < 0.001$).

In contrast, Kaplan–Meier survival analysis of the miRNA signature showed that six miRNAs, hsa-miR-652-5p, hsa-miR-193b-5p, hsa-*miR-129*-5p, hsa-miR-143-5p, has-miR-496, and hsa-miR-7-1-3p, were good predictors of overall survival in bladder urothelial carcinoma. BC T-stages 1–4 imply cancer progression in surrounding tissue layers, from connective tissue underlying the bladder (T1) to TCC (which were 90% of BC cases), 75%–85% of which are nonmuscle invasive (Tis/CIS, Ta, and T1).

In prostate cancer patients, *miR-145* deletion increased disease progression and poor survival, according to [67]. High miR-143/145 levels independently predicted superficial tumor growth and poor muscle-invasive patient survival [68]. In most cancers, *miR-145* is downregulated. *miR-145* may affect Akt and KRas pathway tumorigenicity in the cell microenvironment. Moreover, *miR-145* is upregulated by P53 and FoxO transcription factors [69-71]. Then, *miR-145* silences c-Myc, causing apoptosis and cell cycle arrest. *miR-145* increased pi53 pathway activation and p53 transcriptional targets BBC3 (PUMA) and CDKN1A (P21), suggesting a tumor-suppressor loop [72]. RREB1 represses *miR-145* for KRas-induced oncogenic cell transformation [73].

Screening and therapeutic follow-up should be replaced with routine *miR-129* and *miR-145* deployment. Urine or plasma tests can detect noninvasive symptoms. Furthermore, we need more participants to confirm our findings. The miRNA signature may improve survival-associated miRNA research and BLC miRNA target-based therapies.

In conclusion, human BC malignancies depended on *miR-145* and *miR-129* for carcinogenesis, progression, histological pattern, grade, CT tumor size, recurrence, survival, and mortality. It may also be a future BC biomarker used for screening, prognosis, and identifying treatment targets.

Author Contribution Statement

All authors contributed to the study idea and design, experimental work, paper writing, editing, and revision.

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Ethical approval and consent to participate

Studies Including Human Subjects. The Declaration of Helsinki was followed in the conduct of the study. The Menoufia University Faculty of Medicine Ethics Committee approved the study procedure, and all subjects supplied written informed permission.

Conflicts of interest

The study authors affirm that no conflicts of interest could influence the findings.

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