Al-Rafidain J Med Sci. 2024;7(1):215-220.
DOI: https://doi.org/10.54133/ajms.v7i1.1229

A J M S
Online ISSN (2789-3219)

Research Article

Relationship between Human Telomerase Reverse Transcriptase Gene and Some microRNAs Expression Levels in Patients with Bladder Cancer

Sura Mouaid Abbas[1]*, Maysaa Abdul Razzaq Dhahi[2]
[1]*Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq;* [2]*Department of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq*
Received: 18 July 2024; Revised: 23 August 2024; Accepted: 27 August 2024

Abstract

Background: Bladder cancer (BC) is the fourth most prevalent cancer among the top ten malignancies, and human telomerase reverse transcriptase (*hTERT*) played a role in its pathogenesis. ***Objective***: To investigate the link between *hTERT* expression levels and miRNA-29c, miRNA-125, miRNA-141, miRNA-145, and miRNA-205 expression levels in BC patients. ***Methods***: A total of 149 tissue biopsies and/or urine samples were collected from patients with urinary tract complications, including BC patients, as well as patients who served as negative controls for BC (negative cystoscopy, prostate cancer patients), and healthy people. To measure the *hTERT* gene expression level, total RNA was extracted and reverse-transcribed to cDNA. Then, quantitative real-time PCR was performed using specific primer sets. ***Results***: *hTERT* expression levels in BC patients were significantly higher; however, there was no statistically significant difference between the grade of non-muscle invasive BC or the stages of muscle invasion in urine or tissue biopsy samples. The expression of *hTERT* and miRNAs (miRNA-29c, miRNA-125, miRNA-141, miRNA-145, and miRNA-205) genes in urine and tissue biopsies was significantly correlated. BC patients had upregulated *hTERT* expression levels in tissue biopsies and urine samples but lower expression levels of miR-141 and miR-205 compared to negative cystoscopy patients. ***Conclusions***: The combination of miRNA and *hTERT* expression levels may serve as a good prognostic marker for predicting BC in both urine and tissue.

Keywords: Bladder cancer, Gene expression levels, *hTERT*, miRNAs.

العلاقة بين جين النسخ العكسي للتيلوميراز البشري وبعض مستويات التعبير عن الحمض النووي الريبي الميكروي لدى مرضى سرطان المثانة

الخلاصة

الخلفية: سرطان المثانة هو أكثر انواع السرطانات انتشاراً ويحل بالمستوى الرابع ضمن اول 10 انواع من السرطانات الخبيثة. أن جين النسخ العكسي للتيلوميراز البشري *hTERT* دوراً كمسبب لسرطان المثانة. **الهدف:** التحقق عن العلاقة بين مستويات التعبير من *hTERT* و miRNA-29c و miRNA-125 و miRNA-141 و miRNA-145 و miRNA-205 لدى مرضى سرطان المثانة. **الطريقة:** جمعت 149 عينة (خزعة نسيجية أو/و عينة ادرار) من مرضى لديهم مضاعفات في الجهاز البولي، شملت مرضى سرطان المثانة بالاضافة الى مرضى كعامل سيطرة سلبي لسرطان المثانة (ناظور المثانة السلبي ومرضى سرطان البروستات) والاصحاء. من أجل تقدير التعبير الجيني لـ*hTERT* تم استخلاص RNA الكلي وتحويله الى cDNA. تم إجراء التقدير الكمي بواسطة Real time-PCR باستخدام بادئات محددة.**النتائج**: كانت مستويات تعبير *hTERT* في مرضى BC أعلى بكثير. ومع ذلك، لم يكن هناك فرق ذو دلالة إحصائية بين درجة BC غير الغازي للعضلات أو مراحل غزو العضلات في عينات الأدرار و خزعة الأنسجة. ارتبط التعبير عن جينات *hTERT* و mRNAs (miRNA-145, miRNA-205 miRNA-125, miRNA-141,) في خزعات الأنسجة وعينات الأدرار بشكل كبير. كان لدى مرضى BC مستويات تعبير *hTERT* عالية في خزعات الأنسجة وعينات الأدرار ولكن مستويات تعبير أقل من miR-141 و miR-205 مقارنة بمرضى تنظير المثانة السلبي. **الاستنتاجات:** دمج التعبير الجيني للحامض النووي الريبوزي الدقيق مع التعبير الجيني لل *hTERT* من الممكن ان يكون اداة واعدة للتنبؤ بسرطان المثانة في عينات الادرار والخزعة النسيجية.

* ***Corresponding author***: Sura M. Abbas, Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq; Email: sura.mo.a@uomustansiriyah.edu.iq
Article citation: Abbas SM, Dhahi MA. Relationship between Human Telomerase Reverse Transcriptase Gene and Some microRNAs Expression Levels in Patients with Bladder Cancer. *Al-Rafidain J Med Sci*. 2024;7(1):215-220. doi: https://doi.org/10.54133/ajms.v7i1.1229

INTRODUCTION

Bladder cancer, or transitional cell carcinoma (TCC), is the second most common genitourinary tract malignancy and the third most common cause of death among people with genitourinary tumors, with a recurrence rate of BC ranging from 50 to 70%. A majority (80%) of the cases present with non-muscle-invasive papillary tumors, which have a much more adequate prognosis [1,2]. Human telomerase reverse transcriptase (*hTERT*) maintains the length of the telomere. Normal human cells inhibit telomerase activity, whereas over 85% of malignant cancer cells reactivate and overexpress it, enabling them to survive and proliferate uncontrollably. This activity is responsible for telomere maintenance in the majority of human cancers, but it is absent in most normal somatic tissues. Telomerase has been described as a

potential biomarker and a promising therapeutic target for cancer [3-7]. MicroRNAs, also referred to as noncoding RNAs, are a class of small, single-stranded RNA molecules that have endured through evolution and play a crucial role in the epigenetic regulation of numerous genes' expression. A miRNA molecule in humans, which typically has a length of 19–25 nucleotides, regulates a variety of biological processes, including angiogenesis, migration, apoptosis, oncogenesis, and cell division [8,9]. If there is a change in the transcriptional level of miRNA due to changes in miRNA biogenesis, polymorphisms, epigenetic changes, mutations in the genes that code for these miRNAs, or chromosomal abnormalities, it could lead to cancer. These can also happen concurrently, modulating the expression of miRNAs [10]. The aim of this study was to find out if there is an association between the level of *hTERT* expression and the risk of getting bladder cancer, the stage of muscle invasion, and the grade of non-muscles invasive BC in patients from Baghdad, Iraq. Additionally, the study aims to explore the correlation between the expression level of *hTERT* and the expression levels of miRNA-29c, miRNA-125, miRNA-141, miRNA-145, and miRNA-205 in BC patients.

METHODS

Study design and setting

The current case-control study included 149 tissue biopsies and/or urine samples collected from subjects recruited or admitted to Al-Imamein Al-Kadhmain Medical City Hospital and Ghazi-Al-Hariri Specialized Surgery Hospital/Medical City Hospital, Baghdad, Iraq, from November 2018 to August 2019.

Sample collection

Classification of subjects and samples included in the current study according to histopathological findings and cystoscopy: tissue biopsies and urine samples were collected from 49 patients (38 male and 11 female) with BC. The age range of patients with BC was (32-87) years. Patients with BC were classified into two main groups: non-invasive BC (Ta and T1) and invasive BC (T2, T3 and T4). As negative controls for BC, samples were collected from: a) 12 patients with negative cystoscopy (this group includes patients with symptoms of BC such as hematuria in urine but without malignant mass). From patients who had benign mass, 12 urine samples and 4 tissue biopsies were collected. b) Urine and tissue biopsies samples were collected from 4 patients with prostate cancer and adenocarcinoma, which is another type of bladder cancer. c) Urine samples only, 37, were randomly collected from healthy individuals. The classification of BC was according to the cystoscopy finding, which was either high or low grade according to the World Health Organization (WHO) classification criteria 2018 [11]. Tissue biopsy and urine samples from the included subjects were kept at -20°C for molecular analysis. Data were collected from each patient and control group (name, age, gender, smoking habit, previous treatment in patients with recurrent BC). Histopathology findings of each patient were obtained from laboratory reports.

Inclusion criteria

Patients with bladder tumor and negative cystoscopy (no histopathological changes or benign tumor).

Exclusion criteria

Patients missing of histopathological reports (patients' data).

Ethical consideration

This study was approved by ethical committee of the College of Medicine, AL-Nahrain University in Baghdad (No. 328 - Date 11/12/2018)

Quantification of hTERT expression levels

Purified RNA was extracted using an RNA isolation kit (Cat. No. 217004, Qiagen) and mRNA was reverse transcripted using a (Cat. No. 06-17-00100, Solis Bio) kit. Purity and concentration of extracted RNA were estimated using a nanodrops spectrophotometer (Cat. no. AcTGeneNAS-99, USA). For estimation of *hTERT* expression levels, *GAPDH* is considered a housekeeping gene. Master mix was prepared (per one reaction) by mixing 12.5 µl of QuantiTect SYBR Green PCR Master Mix (2x) (cat. No. 218075, Qiagen), 20 nM of forward and reverse primers, Table 1.

Table 1: Primer sequence and amplification program for *hTERT* gene

Genes	Sequences	Annealing temperature	References
hTERT	F- ACT TTG TCA AGG TGG ATG TGA CGG R- AAG AAA TCA TCC ACC AAA CGC AGG	Initial activation step at 95º C for 10 min. Denaturation 94ºC for 10 sec. Annealing 67ºC for 1 min and Extension72 ºC for 15 sec 35 cycle	[12]
GAPDH	F -GAG TCA ACG GAT TTG GTC GT R -TTG ATT TTG GAG GGA TCT CG	Initial activation step at 95º C for 10 min. Denaturation 94ºC for 10 sec. Annealing 52 ºC for 1 min and Extension72 ºC for 15sec 35 cycle	

RNase-free water was added until 22.5 µl. Then, cDNA (75 ng/reaction) was added to the reaction tube. The template control (NTC) tube was prepared, which contains all PCR master mix components but instead of cDNA, 2.5 µl of nuclease-free water was added. Reaction tubes were transferred to a thermal cycler (TC-3000, Bio-Rad), which was programmed as mentioned in Table 1. The relative method was used to calculate fold changes of gene expression level [13]. Note: Another primer set was used to determine

hTERT expression levels [14], but was excluded due to failure of amplification, even after a series of optimizations for primer concentration and amplification conditions.

Relationship between hTERT expression level and selected microRNAs

In the current study, the expression levels of the five studied miRNAs (miRNA-29c, miRNA-125, miRNA-141, miRNA-145, and miRNA-205) in urine and/or tissue biopsies samples from our previous study were used to compare *hTERT* expression levels in samples from the included subjects [15].

Statistical analysis

Quantitative variables were expressed as mean, standard deviation (SD), whereas categorical variables were expressed as number and percentage. The 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 an expected count less than 5 and the Fisher exact test when a cell or more contained an observed value of zero. Values with $p<0.05$ are considered significantly different.

RESULTS

Table 2 presents the gender, age, cancer stage, and classification of bladder cancer patients according to cancer recurrence. The present investigation demonstrated a significant increase in the expression level of *hTERT* at a significance level of $p<0.05$ in both tissue biopsies and urine samples obtained from individuals diagnosed with bladder cancer.

Table 2: Distribution of patients with different stages of bladder cancer according to recurrence

Stage of cancer	T1 n(%)	T2 n(%)	T3 n(%)	T4 n(%)
Patients with BC without recurrence	25/39(64.1)	9/39(23)	3/39(7.6)	2/39(5.1)
Patients with recurrent BC	7/10(70)	2/10(20)	-	1/10(10)
Total			49 patients	

n: number; NMI (pTa and pT1): low-grade tumor or non-muscle invasive; (MIBC) (T2, T3, T4): high-grade tumors or Muscle-invasive cancers.

The fold change of gene expression for *hTERT* was examined in various phases of muscle invasion and grades of non-muscle invasive bladder cancer in both urine and tissue samples. The analysis of the interquartile range and Wilcoxon test (Table 3) indicated that there was no statistically significant alteration ($p>0.05$).

Table 3: Fold change of gene expression level of *hTERT* in urine and tissue biopsies samples among different groups of studied subjects

Gene	Sample	Fold change	Negative cystoscopy	Prostate cancer	Noninvasive BC	Invasive BC	Recurrent BC
hTERT	Urine	Upregulation	8(80)	2(100)	10(58.8)	10(83)	3(42.8)
		Downregulation	2(20)	0	7 (41.2)	2(17)	4(57.2)
		Median (IQR)	22.03(---)	15.0 (---)	16.03(13.67)	15.0(7.22)	15.81(13.98)
		Range	14.00-28.09	14.49-15.50	13.3-31.0	13.3-29.0	14.0-29.0
		Total samples	10	2	17	12	7
	Tissue	Upregulation	2(50)	2(66.7)	16(66.6)	4(40)	5(83)
		Downregulation	2(50)	1(33.3)	8(33.4)	6(60)	1(17)
		Median (IQR)	24.83 (---)	22.64 (---)	19.30(15.53)	23.2(13.23)	16.20 (5.73)
		Range	18.20-32.60	15.27-30.0	14.00-30.56	13.6-31.0	14.68-23.53
		Total samples	4	3	24	10	6
		p	0.593 W	0.655 W	0.778 W	0.260 W	0.686 W

Values are expressed as frequencies, percentages, and ranges. IQR: inter-quartile range, W: Wilcoxon test; CA: Cancer.

Regarding the findings indicated that 83% (10 out of 12) of urine samples obtained from individuals diagnosed with invasive bladder cancer exhibited an increased expression level of *hTERT*.

The Kruskal-Wallis test was used to evaluate the distribution of fold change in gene expression level of *hTERT* in urine and tissue biopsies (Figures 1 and 2). Boxplot illustrating the results of the Kruskal-Wallis test. The fold change in the gene expression levels of *hTERT* was increased in the urine of individuals diagnosed with bladder cancer.

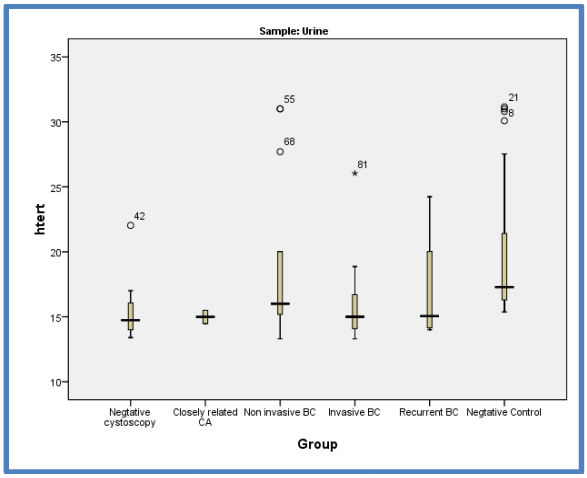

Figure 1: Box -plot of the Kruskal-Wallis for Fold change in gene expression level of *hTERT* in urine samples according to patients group.

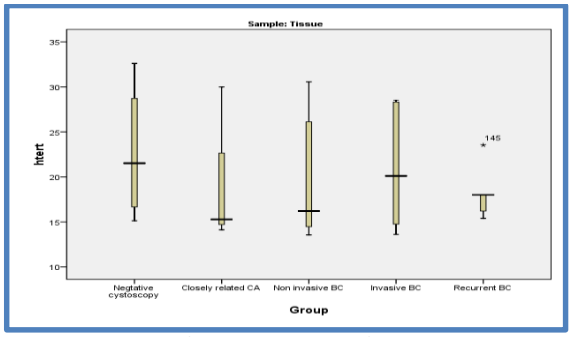

Figure 2: Box -plot of the Kruskal-Wallis for Fold change in gene expression level of *hTERT* in tissue biopsies samples according to patients group.

The study also found a disparity in the median *hTERT* expression level between samples from individuals with bladder cancer and those without a positive bladder cancer cystoscopy, suggesting a reduced upward trend in expression level. There were no notable variations in *hTERT* expression levels in urine among various cancer grades and stages. The expression level of the *hTERT* gene was shown to be more variable in the noninvasive and recurrent groups of bladder cancer patients compared to other groups. This was demonstrated by a box plot of the Kruskal-Wallis test, which examined the fold change in gene expression level value of *hTERT* mean area upward increased expression in tissue biopsies from BC patients. The present investigation revealed a statistically significant connection (p<0.01) in the fold change of gene expression level between *hTERT* and the examined microRNAs in both urine samples and tissue biopsy samples. This correlation was determined using the interquartile range and Friedman test, as indicated in Tables 4 and 5. Patients diagnosed with BC exhibited a decreased fold change in the gene expression level of miR-205 (p=0.001) and an increased fold change in the gene expression level of *hTERT* (p<0.001) in urine samples, as compared to patients with negative cystoscopy. This information can be found in Tables 4 and 5.

Table 4: Comparison of fold change in genes expression levels between *hTERT*, and studied microRNAs in urine samples

Group	*hTERT*	miR-29c	miR-125	miR-141	miR-145	miR-205	*p**
Negative cystoscopy (*n*= 7)							
Median (IQR)	14.87(3.0)	0.45(1.82)	0.19(7.81)	0.32(13.35)	18.83(22.22)	2.76(24.74)	<0.001
Range	14.0-22.0	0.02-16.13	0.07-14.42	0.04-15.52	0.03-38.72	0.04-25.88	
Prostate cancer (*n*= 2)							
Median (IQR)	15.0(---)	22.15(---)	9.54(---)	0.07(---)	5.95(---)	0.43(---)	<0.001
Range	14.00-16.00	17.90 26.39	5.90-13.18	0.06-0.07	0.14-11.75	0.04-0.82	
Non- invasive BC (*n*= 10)							
Median (IQR)	15.73(7.0)	4.84(17.32)	0.57(0.95)	0.24(0.35)	0.68(8.51)	0.30(5.32)	<0.001
Range	13.00-31.0	0.02-38.37	0.16-11.31	0.04-1.46	0.05-14.67	0.02-32.31	
Invasive BC (*n*= 10)							
Median (IQR)	14.86(3.0)	1.49(19.55)	5.69(17.46)	0.63(2.11)	1.34(13.02)	1.73(7.62)	<0.001
Range	13.0-26.0	0.18-28.28	0.07-43.41	0.27-20.76	0.06-75.32	0.06-18.43	
Recurrent BC (*n*= 4)							
Median (IQR)	15.06(8.0)	0.33(7.81)	13.68(32.32)	0.95(28.74)	3.48(6.01)	0.44(1.02)	<0.001
Range	14.0-24.00	0.07-10.37	0.51-35.88	0.39-38.48	0.49-6.6	0.12-1.28	

n: number of samples; IQR: Inter-quartile range; * Freidman test.

Table 5: Comparison of fold change in gene expression level between *hTERT* and studied microRNA in tissue biopsies samples

Group	*hTERT*	miR-29c	miR-125	miR-141	miR-145	miR-205	*p**
Negative cystoscopy (*n*= 3)							
Median (IQR)	18.2(---)	2.89(---)	0.86(---)	0.56(---)	0.4(---)	0.6(---)	0.001
Range	15.0-25.0	1.87-11.57	0.69-6.44	0.14-0.58	0.41-12.23	0.04-4.64	
Prostate cancer (*n*= 3)							
Median (IQR)	15.27(---)	5.51(---)	15.89(---)	1.02(---)	80.73(---)	3.93(---)	0.001
Range	14.0-30.0	1.95-24.80	4.78-21.95	0.92-36.15	36.38-90.2	1.02-17.93	
Noninvasive BC (*n*= 14)							
Median (IQR)	16.2(7.0)	2.41(8.28)	1.44(3.5)	3.19(10.24)	5.05(29.11)	2.4(11.78)	0.001
Range	14.0-30.0	0.07-28.09	0.04-25.11	0.02-46.72	0.1 -43.96	0.04-41.18	
Invasive BC (*n*= 6)							
Median (IQR)	20.1(14.0)	9.59(31.09)	0.54(16.74)	1.16(2.83)	3.41(11.85)	2.36(7.84)	0.001
Range	14.00-29.0	0.03-57.76	0.06-34.3	0.04-5.05	0.06- 33.01	0.01-15.6	
Recurrent BC (*n*= 4)							
Median (IQR)	18.0(6.0)	0.68(6.48)	0.56(10.12)	1.33(11.72)	0.2(9.91)	0.99(28.34)	0.001
Range	15.0-24.0	0.01-8.41	0.11-13.39	0.04-14.82	0.04-13.2	0.02-37.19	

n: number of samples; IQR: Inter-quartile ranges; * Freidman test.

In general, individuals with BC had decreased alterations in the levels of miR-141 and miR-205 genes in their urine samples. In contrast, those with negative cystoscopy reported lower alterations in the levels of *hTERT* genes in both urine and tissue biopsy samples compared to those with positive results (Table 6).

DISCUSSION

Bladder cancer is a complex disease with different molecular and pathological pathways, reflecting different behaviors depending on the clinical staging of the tumor and molecular type [16]. In the present study, the expression level of *hTERT* in patients with BC showed statistically no significant difference between different cancer grades. The number of samples was reduced in this experiment because 15 samples showed no or low Ct for the housekeeping gene, despite repeating the real-time PCR reactions three or four times, indicating a failed quantification reaction. Similarly, 10 samples showed no Ct value for *hTERT*, but we detected Ct for *GAPDH*, indicating the presence of variants in *hTERT* in these samples from the Iraqi population.

Table 6: Percentage of fold change in gene expression between miRNA and *hTERT* in BC patients

miRNA	Sample	BC Patients *n*(%)	
		Up	Down
miR-29c	urine	23(57.5)	17(42.5)
	tissue	26(62)	16(38)
miR-125	urine	23(56)	18(44)
	tissue	26 (62)	16(38)
miR-141	urine	14(35)	26(65)
	tissue	18(43%)	24(57)
miR-145	urine	20(48)	22(52)
	tissue	26(60)	17(40)
miR-205	urine	15(38)	25(62)
	tissue	22(54)	19(46)
hTERT	urine	23(63.8)	13(36.2)
	tissue	25(62)	15(38)

This study employed two primers for the *hTERT* gene, discarding the first one due to its inability to amplify the *hTERT* gene. In 2007, researchers in China used quantitative SYBER GREEN real-time PCR to look at the level of *hTERT* mRNA in the urine of 26 people with BC (7 at the Ta-T1 stage and 19 at the T2-T3 stage) and 18 non-cancerous control samples (6 patients, 5 with benign prostatic hyperplasia, 3 with bladder stones, and 4 with urinary tract infections). The patients had bladder cancer and the control samples did not have cancer. They found a strong link between the expression level of *hTERT* mRNA in urine samples and both the pathological grade and clinical stage. This led them to believe that the expression level of *hTERT* mRNA could be used as a sign of tumor progression to help with early diagnosis and prognosis of BC [17]. In 2010, a study in Perugia involved 36 patients with BC and 58 healthy control groups. They extracted messenger RNAs from exfoliated cells from bladder washings, and used quantitative real-time PCR assays to investigate the expression levels of three human genes, *hTR*, *hTERT*, and *CKS2*. Their results indicated that *hTERT* and *CKS2* expression levels have a major involvement in the early stages of the BC [18]. In this study, the level of *hTERT* expression was higher in 10 of the 12 urine samples from patients with invasive bladder cancer (83%). This means that estimating the expression level of this gene could be a good prognostic tool in late stage of cancer. In this study, the fold change in the expression level of *hTERT* was significantly higher than the fold change in the expression levels of miR-29c, miR-125, miR-141, miR-145, and miR-205 in both urine and tissue samples. Most tumor suppressor miRNAs down-regulate *hTERT*, whereas oncogenic miRNAs indirectly up-regulate *hTERT* activity by inhibiting genes involved in *hTERT* suppression [19-21]. Dysregulation of specific miRNAs that control *hTERT* gene transcription may result in uncontrolled expression of *hTERT*, potentially leading to cancer. An Egyptian study in 2020 used the quantitative SYBER GREEN Real-Time PCR assay to look into the role of both miR-155 and *hTERT* in TCC-BC (as detection tools for stages or grades of muscle invasion). The study included 30 urine samples from patients with BC and 15 samples from patients with normal urological workup. There was no statistically significant difference in the levels of miR-155 and *hTERT* between the different grades of bladder cancer that did not invade muscle [22].

Conclusion

The *hTERT* expression level serves as a promising non-invasive method for predicting bladder cancer from urine samples. Further work to investigate the exact association with specific miRNA in bladder cancer is strongly recommended.

Conflict of interests

No conflict of interests was declared by the authors.

Funding source

The authors did not receive any source of fund.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Kittler R, Shiang C, Hutchinson R, Kollipara RK, Kapur P, Franto F, et al. Grade progression in urothelial carcinoma can occur with high or low mutational homology: a first-step toward tumor-specific care in initial low-grade bladder cancer. *Oncotarget*. 2018;9(10):9415-9424. doi: 10.18632/oncotarget.24072.
2. Yahyazadeh R, Bashash D, Ghaffari P, Kord S, Safaroghli-Azar A, Ghaffari SH. Evaluation of hTERT, KRT7, and survivin in urine for noninvasive detection of bladder cancer using real-time PCR. *BMC Urol*. 2021;21(1):64. doi: 10.1186/s12894-021-00838-z.
3. Alnafakh RAA, Adishesh M, Button L, Saretzki G, Hapangama DK. Telomerase and telomeres in endometrial cancer. *Front Oncol*. 2019;9:344. doi: 10.3389/fonc.2019.00344.
4. Colebatch AJ, Dobrovic A, Cooper WA. *TERT* gene: its function and dysregulation in cancer. *J Clin Pathol*. 2019;72(4):281-284. doi:10.1136/jclinpath-2018-205653.
5. Dratwa M, Wysoczańska B, Łacina P, Kubik T, Bogunia-Kubik K. TERT-regulation and roles in cancer formation. *Front Immunol*. 2020;11:589929. doi: 10.3389/fimmu.2020.589929.
6. Trybek T, Kowalik A, Góźdź S, Kowalska A. Telomeres and telomerase in oncogenesis. O*ncol Lett*. 2020;20(2):1015-1027. doi: 10.3892/ol.2020.11659.
7. Di Nunno V, Aprile M, Bartolini S, Gatto L, Tosoni A, Ranieri L, et al. The biological and clinical role of the telomerase reverse transcriptase gene in glioblastoma: A potential therapeutic target? *Cells*. 2023;13(1):44. doi: 10.3390/cells13010044.
8. Wen W, Mai SJ, Lin HX, Zhang MY, Huang JL, Hua X, et al. Identification of two microRNA signatures in whole blood as novel biomarkers for diagnosis of nasopharyngeal carcinoma. *J Transl Med*. 2019;17(1):186. doi: 10.1186/s12967-019-1923-2.
9. Smolarz B, Durczyński A, Romanowicz H, Szyłło K, Hogendorf P. miRNAs in cancer (Review of literature). *Int J Mol Sci*. 2022;23(5):2805. doi:10.3390/ijms23052805.
10. Menon A, Abd-Aziz N, Khalid K, Poh CL, Naidu R. miRNA: A promising therapeutic target in cancer. *Int J Mol Sci*. 2022;23(19):11502. doi: 10.3390/ijms231911502.
11. Compérat EM, 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. doi: 10.1016/j.euf.2018.01.003.
12. Chen SM, Tao ZZ, Hua QQ, Liu D, Chi HM, Cai Q. Inhibition of human telomerase reverse transcriptase in hep-2 cells using short hairpin RNA expression vectors. *Arch Otolaryngol Head Neck Surg*. 2006;132(2):200-205. doi: 10.1001/archotol.132.2.200.
13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-

Delta Delta C(T)) method. *Methods*. 2001;25(4):402-408. doi: 10.1006/meth.2001.1262
14. Serrano D, Bleau AM, Fernandez-Garcia I, Fernandez-Marcelo T, Iniesta P, Ortiz-de-Solorzano C, et al. Inhibition of telomerase activity preferentially targets aldehyde dehydrogenase-positive cancer stem-like cells in lung cancer. *Mol Cancer*. 2011;10:96. doi: 10.1186/1476-4598-10-96.
15. Abbas SM, Dhahi MA, Muhammad SH. 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. *Iraqi J Sci*. 2023;64(8):3799-3811. doi: 10.24996/ijs.2023.64.8.7.
16. Batista R, Vinagre N, Meireles S, Vinagre J, Prazeres H, Leão R, et al. Biomarkers for bladder cancer diagnosis and surveillance: A comprehensive review. *Diagnostics (Basel)*. 2020;10(1):39. doi: 10.3390/diagnostics10010039.
17. Xie XY, Yang X, Zhang JH, Liu ZJ. Analysis of *hTERT* expression in exfoliated cells from patients with bladder transitional cell carcinomas using SYBR green real-time fluorescence quantitative PCR. *Ann Clin Biochem*. 2007;44(Pt 6):523-528. doi: 10.1258/000456307782268093.
18. Mezzasoma L, Antognelli C, Del Buono C, Stracci F, Cottini E, Cochetti G, et al. Expression and biological-clinical significance of hTR, *hTERT* and CKS2 in washing fluids of patients with bladder cancer. *BMC Urol*. 2010;10:17. doi: 10.1186/1471-2490-10-17.
19. Eckburg A, Dein J, Berei J, Schrank Z, Puri N. Oligonucleotides and microRNAs targeting telomerase subunits in cancer therapy. *Cancers (Basel)*. 2020;12(9):2337. doi: 10.3390/cancers12092337
20. Borkowska EM, Konecki T, Pietrusiński M, Borowiec M, Jabłonowski Z. MicroRNAs which can prognosticate aggressiveness of bladder cancer. *Cancers (Basel)*. 2019;11(10):1551. doi: 10.3390/cancers11101551.
21. Yik MY, Azlan A, Rajasegaran Y, Rosli A, Yusoff NM, Moses EJ. Mechanism of human telomerase reverse transcriptase (*hTERT*) regulation and clinical impacts in leukemia. *Genes (Basel)*. 2021;12(8):1188. doi: 10.3390/genes12081188.
22. Abdelgawad A, Mosbah A, Eissa L. Expression of microRNA-155 and human telomerase reverse transcriptase in patients with bladder cancer. *Egypt J Bas Appl Sci*. 2020;7(1):315-322. doi: 10.1080/2314808X.2020.1816337.