RESEARCH ARTICLE

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# Exploring The Prevalence of Telomerase Reverse Transcriptase Promoter Mutations in Bladder Cancer Patients and Their Correlation with Tumor Characteristics

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## Abstract

**Objective:** This work aimed to find the incidence of *TERT* promoter mutations in a specific patient population and to analyze their association with various tumor characteristics.**Methods:** This study, conducted from April to November 2023, involved collecting 67 formalin-fixed, paraffin-embedded (FFPE) tissue samples from patients undergoing transurethral bladder resection or radical cystectomy at the Urology Department of Almawaddah Private Hospital, Basra, Iraq. The extraction of DNA was achieved through the use of purification Promega kits. *TERT* gene promoter mutations, C228T AND C250T were determined by Sanger sequencing using an automated DNA sequencer, by Macrogen Corporation – Korea. **Result:** Among 67 bladder cancer patients, valid p*TERT* molecular analysis was completed in 59 cases. Of these, 30 patients (50.85%) were found to have p*TERT* mutations. The most common mutation was C228T, identified in 70% (21/30) of cases, followed by C250T in 33.3% (10/30), with one patient exhibiting both mutations. No significant associations were found between *TERT* mutations and factors such as age, sex, or smoking status. However, these mutations were more frequently observed in low-grade tumors, occurring in 63.3% (19/30) of cases. Additionally, the prevalence of *TERT* mutations differed significantly between non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC), with mutations detected in 60.4% (26/42) of NMIBC cases and 23.5% (4/17) of MIBC cases (p = 0.027). Although a slight difference in mutation frequency was noted between newly diagnosed and recurrent tumors, it did not reach statistical significance. **Conclusion:** this study demonstrates a substantial prevalence of *TERT* promoter mutations particularly the C228T variant in bladder cancer, which was more frequently found in NMIBC compared to MIBC. No correlations were identified between *TERT* mutations and demographic factors such as age, sex, or smoking history.

**Keywords:** *TERT* promoter mutation- bladder cancer- cancer genetics- somatic mutation

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## Introduction

Human telomerase's catalytic component is produced by the telomerase reverse transcriptase (*TERT*) gene, an enzyme crucial for maintaining telomere length [1]. Elevated telomerase activity is recognized as a key feature of many human cancers, and the dysregulation of *TERT* gene expression is central to its cancer-specific activation [2]. Regardless of tumor stage or grade, *TERT* promoter mutations are the most common genetic alterations in urothelial bladder cancer (UBC), occurring in 60–80% of cases. These mutations are linked to higher levels of *TERT* expression and increased telomerase activity [3, 4].

The specific mutations involve C-to-T transitions at nucleotide positions 1,295,228 (C228T) and 1,295,250 (C250T), located upstream from the gene's transcriptional start site. These alterations disrupt normal telomerase regulation, leading to excessive telomerase activity [5]. These mutations, which are thought to increase *TERT* expression by creating new binding sites for transcription factors in the E-twenty-six (ETS)/ELK family, are the first mutations to be identified in a gene promoter region in human malignancies [6].

In bladder cancer, the C228T mutation is present in approximately 50–70% of cases, while the C250T mutation is observed in 8–15%. These mutations are generally mutually exclusive, and they occur at similar rates in both non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) [7]. Additionally, the presence of *TERT* promoter mutations may render

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tumors more immunogenic, potentially improving their response to immunotherapy. This highlights the value of these mutations as biomarkers that could inform treatment strategies [8].

This research is the first investigation in Iraq to evaluate the prevalence of *TERT* promoter mutations in bladder cancer and explore their associations with tumor characteristics, including stage, grade, and recurrence. While previous research has demonstrated the high frequency of these mutations globally, their prevalence and potential clinical implications in the Iraqi population remain unexplored. This research bridges that gap, providing valuable insights into regional genetic variation and offering a foundation for understanding the role of *TERT* promoter mutations in bladder cancer management within this specific demographic. Additionally, it highlights the significance of C228T and C250T mutations in bladder cancer and their potential to serve as biomarkers for diagnostic and therapeutic advancements.

## Materials and Methods

*Patient's characteristics and Tissue Sampling*

This study, conducted from April to November 2023, involved collecting 67 formalin-fixed, paraffin-embedded (FFPE) tissue samples from patients undergoing transurethral bladder resection or radical cystectomy at the Urology Department of Almawaddah Private Hospital, Basra, Iraq. Patients included in the study had histologically confirmed bladder cancer (BC), and those with other types of cancer were excluded. The study protocol received approval from the Institutional Review Board (IRB) at the College of Medicine, Al Nahrain University according to document number 20221171 on April 17, 2023, to get this approval.

All participants gave informed consent after being thoroughly briefed on the study's purpose and objectives. In line with ethical standards, participant confidentiality and anonymity were strictly maintained. The study population's details are outlined in Table 1. Tumor classification followed the TNM staging system, where non-muscle invasive bladder cancer (NMIBC) included stages Ta, T1, and Tis, while muscle invasive bladder cancer (MIBC) included stages T2, T3, and T4, in accordance with World Health Organization (WHO) criteria.

*DNA Extraction*

AmoyDx® FFPE DNA Kit (Spin Column) from Amoy Diagnostics Co was used for DNA extraction from the FFPE tissue samples (Catalog number 361027). Nanodrop spectrophotometer was used to quantify Concentration of DNA and DNA purity was measured through the absorbance ratio at 260/280 nm, where values between 1.8 and 2 indicated pure DNA.

*TERT Mutational Status Analysis*

Out of the 67 patients included in the study, molecular analysis was successfully completed for 59. Sanger sequencing of polymerase chain reaction (PCR) products was employed to detect *TERT* promoter mutations. A 127-base pair segment of the *TERT* promoter was amplified using PCR with the following primers: GAAGGTGAAGGGGCAGGACG (forward) and GGAGCAGCTGCGCTGTCGG (reverse). Each PCR reaction had a total volume of 25 µL, comprising 12.5 µL of PCR Master Mix (Promega) and 1 µL of each primer. The thermal cycling protocol began with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 63°C for 30 seconds, and extension at 72°C for 45 seconds.

*Statistical analysis*

Nominal variables were analyzed using descriptive statistics, with results presented as frequencies and percentages. To assess relationships between variables, the Chi-square test was employed, considering p-values less than 0.05 as indicative of statistical significance.

## Results

*Epidemiological and Clinical Parameters*

There were three female patients and sixty-four male participants in the study. With ages ranging from 36 to 89, the median age at diagnosis was 65. The highest occurrence of bladder cancer was observed in individuals in their 60s, representing 63% of the cases. The disease was predominantly found in males (95.5%). Regarding sample collection, 74.6% of the specimens were obtained through transurethral resection of bladder tumors (TURBT), while 25.4% were from radical cystectomy procedures. Table 1 displays the characteristics of the study samples.

*TERT promoter mutations detection in tissue samples*

Out of the 67 patients with bladder cancer, a successful

Table 1. Characteristics of Patients with Urinary Bladder Cancer

| Parameter | Numbers (%) |
|---|---|
| Age | |
| <60 years | 25 (37.3%) |
| >60 years | 42(62.7%) |
| Gender | |
| Male | 64 ( 95.5%) |
| Female | 3 (4.5%) |
| Tumor stage | |
| ≤PT1 | 48 (71.6%) |
| >PT1 | 19 (28.4%) |
| Tumor grade | |
| Low grade | 36 (53.7%) |
| High grade | 31 (46.3%) |
| Recurrence | |
| Yes | 24 (35.8%) |
| No | 43 (64.2%) |
| Smoking | |
| Smoker | 46 (68.7%) |
| Nonsmoker | 21 (31.3%) |

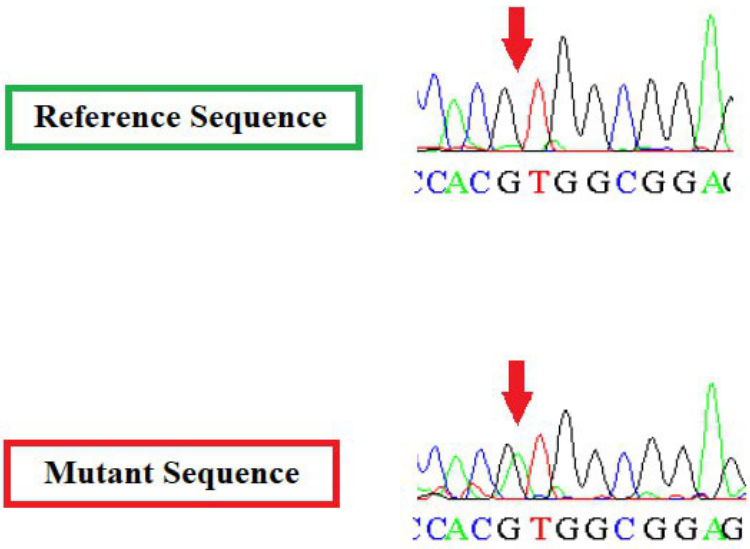


Figure 1. *TERT* Promoter Mutation in Bladder Cancer Patients, detected in DNA sequencing, the C228T mutation identified in the complementary strand as G228A.

molecular analysis of the *TERT* promoter mutation was conducted in 59 cases. Among these, 30 patients (50.85%) exhibited a *TERT* promoter mutation. The C228T mutation was the most frequently observed, present in 70% (21 out of 30) of the cases (Figure 1), followed by C250T, which was found in 33.3% (10 out of 30) Figure 2. One patient had both C228T and C250T mutations.

No significant associations were found between *TERT* mutations and factors such as age, sex, or smoking status. These mutations in the *TERT* gene's promoter region were more commonly observed in low-grade tumors, with a prevalence of 63.3% (19 out of 30). The occurrence of *TERT* mutations differed significantly between NMIBC (26 out of 42, 60.4%) and MIBC (4 out of 17, 23.5%) with a p-value of 0.027. There was also a slight variation in mutation frequency between newly diagnosed and recurrent tumors (p = 0.979) Table 2.

## Discussion

In recent years, significant research has focused on genetic alterations in various cancers, aiming to identify mutations with potential clinical relevance for prognosis, treatment, and follow-up strategies [9]. Among these alterations, hot spot mutations in the promoter region of telomerase reverse transcriptase (*TERT*) have emerged as a new kind of somatic mutation implicated in cancer progression [6]. Numerous studies have investigated *TERT* promoter mutations in various cancers to understand their role in carcinogenesis, prognosis, and treatment response [9].

In this study, the prevalence of *TERT* promoter mutations was found to be 50.85%, consistent with findings from other studies, which reported rates such as 50.33% in bladder cancer (BCa) patients (5) and 56% (1346/2411) in bladder cancers [10]. Other studies have

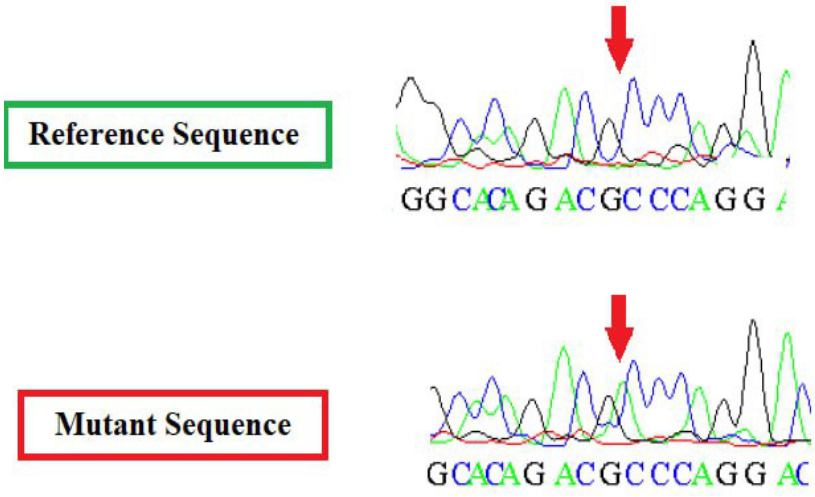


Figure 2. *TERT* Promoter Mutation in Bladder Cancer Patients, detected in DNA sequencing, the C250T mutation identified in the complementary strand as G250A

Table 2. *TERT* Promoter Mutation Frequencies and Their association with Clinical Parameters in Patients with Bladder Cancer

| Parameters | n | Mutation | | C228T | C250T |
|---|---|---|---|---|---|
| | | Mut | WT | | |
| All | 59 | 30 | 29 | 21 | 10 |
| | | 50.85% | 49.15% | 70% | 33% |
| Age | | | | | |
| <65 years | 21 | 12 | 9 | 10 | 2 |
| | | 57% | 43% | 83.30% | 16.70% |
| >65 years | 38 | 18 | 20 | 11 | 8 |
| | | 47.37% | 52.63% | 61.10% | 44.40% |
| | | P-value 0.645 | | | |
| Gender | | | | | |
| Male | 56 | 28 | 28 | 20 | 9 |
| | | 50% | 50% | 71.40% | 32.10% |
| Female | 3 | 2 | 1 | 1 | 1 |
| | | 66.70% | 33.40% | 50% | 50% |
| | | P-value 0.676 | | | |
| Tumor grade | | | | | |
| Low grade | 30 | 19 | 11 | 13 | 7 |
| | | 63.30% | 36.70% | 68.40% | 36.80% |
| High grade | 29 | 11 | 18 | 8 | 3 |
| | | 38% | 62% | 72.70% | 27.30% |
| | | P-value 0.065* | | | |
| Muscle invasiveness | | | | | |
| NMIBC | 42 | 26 | 16 | 18 | 9 |
| | | 60.40% | 39.60% | 69.20% | 34.60% |
| MIBC | 17 | 4 | 13 | 3 | 1 |
| | | 23.50% | 76.50% | 75% | 25% |
| | | P-value 0.027* | | | |
| Recurrence | | | | | |
| Yes | 21 | 11 | 10 | 8 | 3 |
| | | 52.40% | 47.60% | 72.70% | 27.30% |
| No | 38 | 19 | 19 | 13 | 7 |
| | | 50% | 50% | 68.40% | 36.80% |
| | | P-value 0.979 | | | |
| Smoking | | | | | |
| Smoker | 40 | 23 | 17 | 15 | 9 |
| | | 57.50% | 42.50% | 65.20% | 39.10% |
| Non-Smoker | 19 | 7 | 12 | 6 | 1 |
| | | 37% | 63% | 85.70% | 14.30% |

shown higher frequencies, such as 60% [11], 65.4% [1], and up to 76.8% of urothelial bladder cancers harboring *TERT* promoter mutations [6, 12].

Previous research generally suggests no significant difference in the incidence of *TERT* mutations by age. Although our study showed a higher number of mutations in patients under 60 years old, this difference was not statistically significant. Similar results were reported in other studies [9, 13, 14]. For instance, Siraj et al. found no significant difference in mutation rates between age groups [15], while studies by Wu et al. and Giedl et al. reported a significantly higher mutation rate in older patients, with rates of 60% and 84.8% compared to 37.5% and 57.6% in younger patients, respectively [16, 17].

This study found no significant association between mutations in *TERT* promoter region and gender ($p = 0.676$), aligning with findings from prior research [9]. Several studies involving bladder cancer patients with a mean age of 65–75 years also reported no significant relationship between gender and *TERT* mutation rates [6, 13, 15].

However, Wu et al. observed a tendency for higher mutation rates in men compared to women [16].

*TERT* promoter mutations were more prevalent in low-grade tumors than high-grade ones (63.3% vs. 38%). This is consistent with findings by [18], who found that low-grade Ta tumors have higher rate of *TERT* promoter mutations than high-grade or carcinoma in situ (CIS) tumors, though this difference was not statistically significant. Conversely, other studies, such as [11], found that *TERT* mutations were more common in high-grade tumors (65.11%; 28/43). However, some studies found no significant difference in mutation rates between tumor grades [9, 15].

Multiple studies suggest that *TERT* promoter mutations are related with worse survival outcomes and higher recurrence rates in urothelial bladder cancers. However, this study found no significant difference in mutation rates between newly diagnosed and recurrent tumors ($p = 0.979$), which is consistent with other research [6, 9]. Although some studies observed a trend toward higher recurrence rates in tumors with *TERT* mutations, these findings were not statistically significant [9].

Regarding tumor stage, *TERT* mutations were shown to be more prevalent in non-muscle-invasive bladder cancer (NMIBC) (60.4%) in this study, with a statistically substantial correlation exists between mutation rate and tumor stage ($p = 0.027$). This result aligns with previous studies, such as Pietzak et al., who reported that *TERT* mutations were the most common mutation in NMIBC, present in 73% of cases [19]. In contrast, Wu et al. found that compared to early-stage NMIBC,*TERT* expression was higher in invasive and advanced bladder [16].

Smoking remains a significant risk factor for bladder cancer. In this study, the incidence of *TERT* promoter mutations was higher in smokers than non-smokers (57.5% vs. 37%), though this difference was not statistically significant. These findings are consistent with prior research that also found no significant difference in *TERT* mutation rates between smokers and non-smokers [9].

The most common mutation detected was C228T, present in 70% of cases, followed by C250T, which mirrors the findings of Allory et al.[6], who reported C228T as the most frequent mutation at the −124 nucleotide position These results align with earlier studies indicating that the C228T and C250T mutations occur in 50–70% and 8–15% of bladder cancer cases, respectively [1, 20, 21]. The C228T mutation is especially noteworthy because it changes healthy bladder stem cells into cells that initiate tumors. This mutation, which is found in the *TERT* promoter region, drives tumor formation, and is considered a critical factor in tumor progression [22]. Both C228T and C250T mutations are mutually exclusive and occur with similar frequencies in both NMIBC and MIBC [7]. Monoallelic *TERT* promoter mutations create a binding site for the ETS transcription factor, which activates h*TERT* transcription and telomerase, contributing to tumor progression and relapse.

The ability of the C228T mutation to change healthy bladder stem cells into cells that initiate tumors makes it particularly notable. This mutation, which is found in the *TERT* promoter region, is thought to be a key contributor to the development of tumors.

In conclusion, according to this study, *TERT* promoter mutations are significantly more common in bladder cancer, with C228T being the most prevalent mutation, followed by C250T. *TERT* mutations were more frequent in NMIBC compared to MIBC, with a statistically significant difference in their distribution. Additionally, the low grade tumor had a higher prevalence of *TERT* mutations. However, no significant associations were found between *TERT* mutations and factors such as age, sex, or smoking status. Although mutation rates between newly diagnosed and recurrent tumors showed minor differences, these were not statistically significant. These findings could inform future clinical trials targeting *TERT* promoter mutations in bladder cancer.

## Author Contribution Statement

Hanan H. Ramadhan: collecting the data, writing the paper, and review it. Omar F. Abdul-Rasheed: Conception and design of study. Safaa A. Alhamedi, participating in the collecting of sample, review the paper and editing. Safaa G. Mezban: participating in the collecting of sample. This authorship contribution statement is signed by the corresponding author on behalf of all the listed authors in the manuscript..

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*Approval*

The study protocol received approval from the Institutional Review Board (IRB) at the College of Medicine, Al Nahrain University.

*Ethical Declaration*

The present study has been done after approved by Research Ethics Committee of the College of Medicine, Al Nahrain University according to document number 20221171 on April 17, 2023.

*Conflict of Interest*

there is no conflict of interest to be declared and the study was funded by the researchers themselves.

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