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ERCC1 rs11615 Single Nucleotide Polymorphism in Bladder Cancer Patients and Safety Outcomes of Cisplatin-Based Chemotherapy

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ABSTRACT

Objectives To investigate the relationship between single nucleotide polymorphism (SNP) of excision repair cross-complementation group 1 (ERCC1) rs11615 and the safety outcomes of cisplatin-based adjuvant chemotherapy in post-cystectomy bladder cancer patients.
Methods This pilot single-center retrospective cohort research study involved 25 patients with muscle-invasive bladder cancer who were admitted to the Urology and Nephrology Center (UNC), Mansoura University, Egypt between January and December 2016. The study tried to reveal the association between SNP rs11615 in ERCC1 gene and the change from baseline characteristics in a median of 96 weeks follow-up in blood, renal, and hepatic biochemical parameters.
Results Univariate analysis of the association between rs11615 and post-chemotherapy changes in uric acid and hemoglobin levels showed a statistically significant relationship.
Conclusion These findings hint at possible genetic influences in response to chemotherapy, necessitating further research to understand the mechanisms and potential clinical implications. This could lead to personalized treatment plans enhancing patient care and outcomes.

Keywords: ERCC1, rs11615, Bladder Cancer, Chemotherapy, Safety Outcomes.

1. INTRODUCTION

Bladder cancer represents a significant public health concern in Egypt, characterized by a higher incidence rate, particularly in rural areas, with a notable predominance in male patients. The average age at diagnosis in Egypt is approximately 58 years, substantially lower than the global median ages of 69 for males and 71 for females. Occupational exposure to hazardous substances such as pesticides and fertilizers, common in agricultural practices, has been strongly correlated with the incidence of bladder cancer in these regions. Additionally, the historically high prevalence of Schistosoma haematobium infection, linked to bilharziasis, has been acknowledged as a contributory risk factor in the etiology of bladder cancer. Notably, recent control measures have successfully reduced schistosomiasis rates, leading to a discernible shift in the histopathology of bladder cancer, with a decline in the dominance of squamous cell carcinoma [1, 2].

The genomic landscape of bladder cancer is complex and heterogeneous, driven by genetic changes that include mutations and activation of oncogenes, inactivation of tumor suppressor genes, and chromosomal rearrangements or

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deletions. These genomic alterations lead to diverse clinical and pathological manifestations of bladder cancer, underscoring the need for comprehensive genomic research to inform diagnosis and treatment strategies [3].

In this genomic context, the ERCC1 gene, specifically the SNP rs11615, has emerged as a focal point of research. ERCC1 plays a crucial role in the nucleotide excision repair pathway, responsible for repairing DNA damage, including that inflicted by chemotherapy agents such as cisplatin. While ERCC1 expression has been proposed as a prognostic biomarker in bladder cancer, indicating potentially better survival outcomes for patients with ERCC1-positive tumors, its utility as a predictive biomarker for chemotherapy response, especially to platinum-based treatments, is not definitively established. This is in part due to variability in research findings, differences in assay methods, and the absence of universally accepted threshold values for ERCC1 expression [4, 5].

The ERCC1 gene also plays a key role in the DNA repair process, specifically in making critical cuts in DNA strands, and has been linked to the development of cancer and the ability to withstand chemotherapy and radiation treatments. The role of ERCC1 in predicting the outcomes for bladder cancer patients undergoing cisplatin chemotherapy remains a matter of debate. The regulation of ERCC1 expression, reportedly influenced by mechanisms such as tyrosine kinase receptors and the process of endothelial-mesenchymal transition, necessitates additional research to better understand its role in chemoradiotherapy resistance and to identify new treatments for advanced and metastatic bladder cancer [6].

Investigations into the ERCC1 rs11615 genotype have revealed its potential to significantly influence the pharmacodynamics of cisplatin therapy, a cornerstone treatment for bladder cancer. Patients with specific variants of this SNP may experience different survival rates and responses to chemotherapy [7]. Not only variations in the ERCC1 genotype, but also its expression [8] have an impact on the therapeutic outcomes of platinum-based chemotherapy, which highlights the importance of incorporating pharmacogenomic data into clinical decision-making processes. Recent research has continued to explore the implications of ERCC1 in bladder cancer, examining its relationship with carcinogenesis and resistance to chemoradiotherapy.

The field of clinical pharmacogenomics is poised to revolutionize the personalization of cancer treatment, including chemotherapy regimens for bladder cancer, by studying genetic variations such as those in ERCC1. By tailoring treatment to the individual's genetic profile, there is significant potential to enhance treatment efficacy and reduce adverse effects. However, the inherent multifactorial nature of cancer and the complexity of genetic interactions present challenges in the clinical application of pharmacogenomics [9]. The rapidly growing field of pharmacogenomics aims to refine cancer therapeutics to the genetic profile of each individual, and the ERCC1 rs11615 genotype stands as a promising candidate in this pursuit. As studies continue to elucidate the complex interplay between genetic markers and drug efficacy, incorporating such genomic data holds the promise of optimizing therapeutic strategies, improving outcomes, and mitigating the adverse effects of treatment in bladder cancer patients [10, 11].

This study aimed to investigate the relationship between the SNP rs11615 in the ERCC1 gene and the safety and outcomes of cisplatin-based adjuvant chemotherapy in post-cystectomy bladder cancer patients. By integrating recent genomic insights with the historical and environmental context of bladder cancer in Egypt, we seek to contribute to the personalized treatment approaches that can lead to improved patient outcomes in this challenging disease landscape.

2. METHODS

2.1. Patient Selection and Study Design

This study was structured as a pilot single-center, retrospective cohort analysis. A cohort of 25 patients with muscle-invasive bladder cancer diagnosed with stages T2-T4 was randomly selected using a random number generator. Those patients were admitted to the Urology and Nephrology Center (UNC), Mansoura University, Egypt between January and December 2016. All patients had undergone cystectomy and were subsequently treated with adjuvant chemotherapy as a combination of cisplatin (70 mg/m^2 on day 2) and gemcitabine (1000 mg/m^2 on days 1,8 and 15) repeated every 28 days. Notably, none had received neoadjuvant chemotherapy prior to cystectomy. The primary outcome of concern was a change from baseline in blood, renal, and hepatic laboratory values.

2.1.1 Inclusion criteria

To be included in the study, BC patients who had undergone cystectomy should have a high-risk, muscle-invasive disease with pathological stage T2 – T4 and have received cisplatin-gemcitabine (CG) adjuvant chemotherapy as well as had not received any prior neo-adjuvant chemotherapy.

2.1.2 Exclusion criteria

Patients were excluded from the current study if they were receiving less than four cycles of adjuvant chemotherapy, presenting with grade 2 or higher neuropathy, exhibiting clinical signs of New York Heart Association (NYHA) class III or higher heart failure, having an Eastern Cooperative Oncology Group (ECOG) performance status greater than grade 2, or lost to follow-up.

2.2 Ethical Considerations

The research protocol adhered to the ethical standards of the institutional Review Board (IRB) committee of the Faculty of Pharmacy, Mansoura University, and complied with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all individual participants involved in the study before admission to surgery.

2.3 Genotyping Analysis

For the genotyping of the ERCC1 rs11615 polymorphism, peripheral blood samples were used to extract DNA from the leukocytes using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's guidelines. For SNP genotyping, we utilized the TaqMan® Genotyping Master Mix and TaqMan® SNP Genotyping Assays from Thermo Fisher Scientific, targeting specific SNP rs11615. The genotyping assay included a VIC®-labeled probe for the reference allele, a FAM®-labeled probe for the variant allele, and primers for amplification. The reaction mixture for each sample comprised 5.0 µL of TaqMan® Genotyping Master Mix, 0.25 µL of the respective 20x TaqMan® SNP Genotyping Assay, 2.0 µL of genomic DNA, and 2.75 µL of nuclease-free water, totaling a 10 µL volume. The real-time PCR cycling parameters were set as follows: an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 92°C for 15 seconds for denaturation and 60°C for 1 minute for annealing and extension. Post-PCR, the fluorescence data were analyzed using the real-time PCR system's software, plotting FAM against VIC fluorescence to determine the genotype of each sample as homozygous for the reference allele, homozygous for the variant allele, or heterozygous. Allelic discrimination plots were derived from the real-time PCR assay.

2.4 Statistical Evaluation

Baseline characteristics were systematically tabulated (**Table 1**) and explored for correlations with alterations in biochemical markers. The analysis employed the Kruskal-Wallis test to evaluate non-normally distributed variables, with subsequent Dunn's post hoc testing for detailed pairwise comparisons. Conversely, ANOVA was utilized for the assessment of normally distributed variables. Statistical significance was determined at P-values less than 0.05, employing a two-sided approach to testing. All statistical procedures were executed using STATA 17 software (StataCorp LLC, College Station, TX, USA).

3. RESULTS

3.1. Patients' characteristics

Table 1 represents the baseline characteristics of the study participants. The average age was 60.8 years, with the majority being male (88%). Body mass index (BMI) varied widely among participants, with a median of 28.86, indicating a predominantly overweight cohort. The median follow-up period for this cohort was 96 weeks. Biochemical parameters such as creatinine and uric acid levels varied within expected ranges, demonstrating a median of 0.9 and 5.2 mg/dL, respectively. Hematological parameters showed a median white blood cell (WBC) count of 9.8 $x10^9$/L and mean hemoglobin (Hb) levels of 12.06 g/dL, reflecting the general health status of the population before undergoing chemotherapy.

Table 1. Baseline characteristics of patients.

Parameters	Values
Age, mean ± SD	60.8 ± 7.35
Male gender, n/total (%)	22/25 (88.0%)
BMI, median (IQR) (kg/m^2)	28.86 (24.77, 31.96)
Weight, median (IQR) (kg)	77.5 (70, 88)
Height, median (IQR) (cm)	165.5 (161.5, 171.5)
RBG, median (IQR) (mg/dL)	154 (90 - 224)
Blood parameters	
Hemoglobin, mean ± SD (g/dL)	12.06 ± 1.389
Hematocrit, mean ± SD (%)	36.916 ± 4.324
Platelet (Plt), median (IQR) ($x10^9$/L)	270 (218 - 331)
WBC, median (IQR) ($x10^9$/L)	9.8 (8.5 - 13.7)
RBC, mean ± SD ($x10^{12}$/L)	4.48 ± 0.753
Liver function	
Bilirubin, median (IQR) (mg/dL)	0.5 (0.4 - 0.6)
Albumin, mean ± SD (g/dL)	3.964 ± 0.352
ALT, median (IQR) (U/L)	18 (11 - 24)
AST, median (IQR) (U/L)	17 (12 - 23)
Alkaline Phosphatase, median (IQR) (U/L)	77.5 (64 - 91)
Kidney function	
Serum creatinine, median (IQR) (mg/dL)	0.9 (0.8 - 1.2)
Uric Acid, median (IQR) (mg/dL)	5.2 (4.6 - 6.2)
Electrolytes	
Sodium, mean ± SD (mmol/L)	137.34 ± 3.48
Potassium, median (IQR) (mmol/L)	4.1 (3.8 - 4.25)
Follow-up duration in weeks, median (IQR)	96.3 (41.9 - 232.5)

BMI: Body Mass Index, RBG: Random Blood Glucose, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, WBC: White Blood Cell count, RBC: Red Blood Cell count, Hb: Hemoglobin, Hct: Hematocrit, Plt: Platelets.
Normally distributed data were described as mean ± SD, while non-normally distributed data were described as median (IQR)

3.2. Allelic discrimination

Allelic discrimination derived from the real-time PCR assay is represented in **Figure 1**. The genotype distribution was as follows: 4 patients (16%) were identified with the C/C genotype, 14 patients (56%) exhibited the C/T

genotype, and 7 patients (28%) were characterized by the T/T genotype.

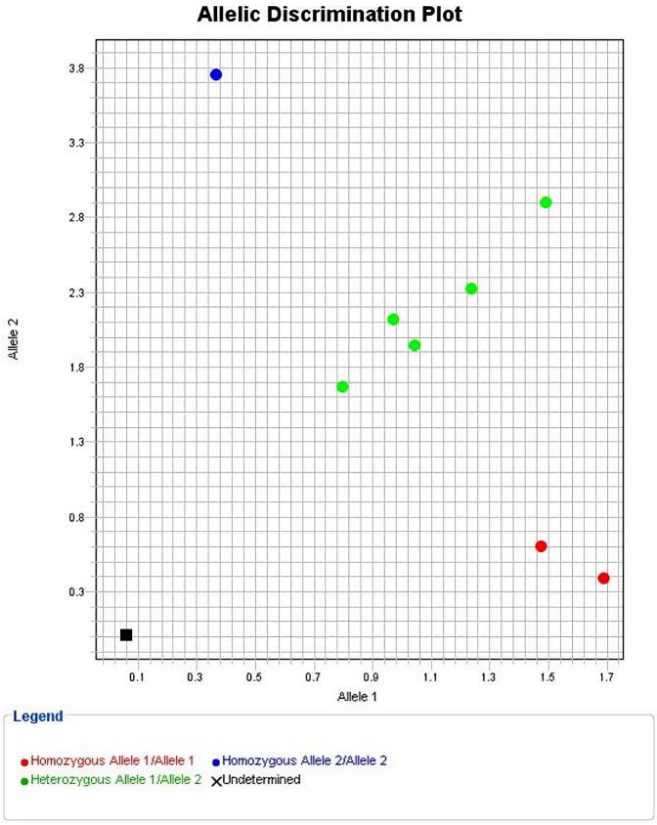

Figure 1. rs11615 allelic discrimination plot.

3.3 ERCC1 rs11615 genetic polymorphism and safety outcomes

As shown in **Table 2**, the univariate analysis of changes in biochemical markers post-chemotherapy, stratified by the specified SNP, revealed several significant associations. For uric acid levels, Dunn's pairwise comparison revealed statistically significant differences when comparing the T/T genotype against both C/C and C/T genotypes. Specifically, individuals carrying the T/T genotype exhibited a median decrease in the uric acid levels (-0.3, interquartile range [IQR] -0.8 to 0.05). On the contrary, increases in the uric acid levels were observed in those with C/C (2.45, IQR 0.68 to 7.9) and C/T genotypes (3.1, IQR 0.4 to 5.4). This suggests a significant protective influence of the T/T genotype on reducing uric acid levels compared to the other genotypes.

Moreover, post-chemotherapy hemoglobin levels were significantly associated with rs11615 genotypes. The C/T genotype showed a milder decline in hemoglobin levels (-1.8, IQR -2.73 to -1.1), as compared to the more pronounced reductions observed in patients with C/C (-3.35, IQR -3.7 to -2.93) and T/T (-3.6, IQR -4.5 to -1.9) genotypes (*P*=0.029). This indicates a potential protective effect of the C/T genotype against the decline in hemoglobin levels observed in other genotypes.

4. DISCUSSION

Variations in patient responses to platinum-based chemotherapy, in terms of toxicity and effectiveness, can often be attributed to genetic differences. The field of pharmacogenetics offers insights into predicting such clinical outcomes based on individual genetic profiles [12, 13]. In line with this, the primary objective of our study was to explore potential links between ERCC1 SNP rs11615 and the change from baseline in biochemical markers determinant of adverse drug reactions in bladder cancer patients following the administration of cisplatin-gemcitabine adjuvant chemotherapy. To the best of our knowledge, this is the first study to assess this kind of association in this subset of patients. This approach allows us to explore the relationship between genetic predispositions and the physiological response to chemotherapy.

The current investigation into the effects of the rs11615 polymorphism on post-chemotherapy biochemical marker changes yielded insightful findings, particularly regarding uric acid and hemoglobin levels. We observed that individuals with the T/T genotype experienced a median decrease in uric acid levels (-0.3, interquartile range [IQR] -0.8 to 0.05), in stark contrast to the increases seen in those carrying the C/C (2.45, IQR 0.68 to 7.9) and C/T genotypes (3.1, IQR 0.4 to 5.4), with statistical significance (P=0.04). This suggests a unique response in uric acid metabolism among patients with the T/T genotype when compared to their C/C and C/T counterparts. Moreover, our analysis further revealed significant variations in hemoglobin levels post-chemotherapy, associated with rs11615 genotypes. Specifically, the C/T genotype was linked to a milder decrease in hemoglobin levels (-1.8, IQR -2.73 to -1.1), as opposed to the more pronounced reductions observed in patients with C/C (-3.35, IQR -3.7 to -2.93) and T/T (-3.6, IQR -4.5 to -1.9) genotypes (P=0.029). This indicates a potential protective effect of the C/T genotype against the decline in hemoglobin levels observed in other genotypes.

The analysis of the effects of rs11615 polymorphism on post-chemotherapy uric acid and hemoglobin levels illustrates the role that genetics play in determining treatment outcomes for bladder cancer patients. Notably, the research highlights a differential response based on genetic makeup, where individuals with the C/T genotype are shown to have a diminished decline in hemoglobin levels post-chemotherapy, demonstrating a genetic resilience against chemotherapy-induced anemia. This observation suggests that the C/T genotype could serve as a protective factor, potentially reducing the incidence of complications such as fatigue and compromised oxygen delivery, thereby facilitating a better tolerance to chemotherapy and improving overall treatment success. In contrast, the T/T genotype is associated with a unique metabolic response leading to a decrease in uric acid levels following chemotherapy, distinguishing these individuals from those with C/C and C/T genotypes, who experienced increases.

Table 2. Associations Between rs11615 Genotypes and Changes in Biochemical Markers.

Outcome measure	Genotype	Change in outcome	P-value
Change in RBG, mean ± SD	C/C	75.5 ± 108.5	0.23
	C/T	-22.14 ± 126.11	
	T/T	-49.33 ± 78.64	
Change in Hb, median (IQR)	C/C	-3.35 (-3.7, 2.93)	**0.03***
	C/T	-1.8 (-2.73, 1.1)	
	T/T	-3.6 (-4.5, 1.9)	
Change in Hct, median (IQR)	C/C	-10.95 (-13.07, 5.45)	0.1
	C/T	-5.1 (-7.83, 2.38)	
	T/T	-9.1 (-13.1, 4.1)	
Change in Plt count, median (IQR)	C/C	-45 (-114.25, 63.25)	0.14
	C/T	-58.5 (-101, 28.5)	
	T/T	-14 (-36, 224)	
Change in WBC, median (IQR)	C/C	-2.5 (-6.63, 0.23)	0.42
	C/T	-3.01 (-6.87, 1.67)	
	T/T	0.29 (-2.38, 1.01)	
Change in RBC, mean ± SD	C/C	-1.2 ± 0.78	0.16
	C/T	-0.47 ± 0.77	
	T/T	-0.95 ± 0.69	
Change in bilirubin, median (IQR)	C/C	0.6 (0.1, 4.2)	0.19
	C/T	0.7 (0.07, 1.73)	
	T/T	0.05 (-0.07, 0.17)	
Change in albumin, mean ± SD	C/C	-1.55 ± 0.45	0.34
	C/T	-1.15 ± 0.61	
	T/T	-0.99 ± 0.66	
Change in ALT, median (IQR)	C/C	3 (-16, 4)	0.85
	C/T	-1 (-13.25, 18.5)	
	T/T	-5.5 (-7, 3.5)	
Change in AST, median (IQR)	C/C	0 (-7, 4)	0.86
	C/T	1 (-4.25, 14)	
	T/T	3 (-7, 16)	
Change in alkaline phosphatase, median (IQR)	C/C	-15 (-97, 160)	0.64
	C/T	54 (14, 122)	
	T/T	34 (22, 46)	
Change in serum creatinine, median (IQR)	C/C	1.8 (0.03, 6.42)	0.30
	C/T	1.21 (0.25, 3.23)	
	T/T	0.1 (0, 1.4)	
Change in uric acid, median (IQR)	C/C	2.45 (0.68, 7.9)	**0.04***
	C/T	3.1 (0.4, 5.4)	
	T/T	-0.3 (-0.8, 0.05)	
Change in Sodium, mean ± SD	C/C	-3.5 ± 7.59	0.87
	C/T	-1.7 ± 5.11	
	T/T	-1.57 ± 8.04	
Change in potassium, median (IQR)	C/C	0.25 (-0.5, 0.62)	0.54
	C/T	-0.52 (-0.76, 0.38)	
	T/T	-0.2 (-0.8, 0.2)	

* P-value < 0.05. Normally distributed data were described as mean ± SD, while non-normally distributed data were described as median (IQR).
Means between SNP groups were compared using ANOVA test and Medians were compared using Kruskal Wallis test, followed by post hoc Dunn's test.

This aspect is particularly significant for patients with pre-existing conditions such as gout or chronic kidney disease, as it implies a reduced risk of exacerbating hyperuricemia-related complications. This genotype-specific insight emphasizes the critical need for preemptive genetic screening to tailor patient management effectively, enabling healthcare providers to predict and address potential adverse effects more accurately.

Adopting genetic testing into the routine management of bladder cancer could markedly refine the personalization of treatment protocols. This genetic foresight allows for a proactive approach to mitigating chemotherapy toxicity and enhancing drug efficacy, thereby elevating patient care quality, enhancing quality of life, and possibly improving survival outcomes. Adopting a precision medicine approach requires integrating individual genetic variations into the planning and monitoring of chemotherapy regimens. This emphasizes the value of genetic data in enhancing cancer treatment.

This study has several strengths such as the meticulous methodology that maintained our internal validity. In addition, we restricted our study to patients with no prior exposure to neoadjuvant chemotherapy and those who received four chemotherapy cycles to exclude confounding by indication. This study highlights the importance of personalized medicine in bladder cancer treatment, aligning with weak evidence levels categorized by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Pharmacogenomics Knowledgebase (PharmGKB) [14, 15].

This study has some limitations including the small sample size and the retrospective nature with its inherited limitations. However, it was designed as a pilot study before we proceeded to the main research question. So, the results need to be interpreted with caution and cannot be generalized, but only used to provide an insight into the broader research question of the impact of the selected SNP and others in the metabolizing pathways for platinum-based compounds.

5. CONCLUSION

The single nucleotide polymorphism of ERCC1 rs11615 showed significant association with the safety outcomes of cisplatin-based adjuvant chemotherapy in bladder cancer patients, particularly regarding uric acid and hemoglobin levels. Further research, including larger-scale studies and comprehensive meta-analyses, is required to confirm these associations and understand their broader implications. This study adds to the growing recognition of the importance of pharmacogenetics in cancer treatment and underscores the need for personalized approaches in oncology.

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Conflicts of Interest
The authors declare no conflict of interest.

6. REFERENCES

1. Zarzour AH, Selim M, Abd-Elsayed AA, Hameed DA, Abdelaziz MA. Muscle invasive bladder cancer in Upper Egypt: the shift in risk factors and tumor characteristics. *BMC Cancer*. 2008; 8:250.
2. Al Saidi I, Mohamedabugroon A, Sawalha A, Sultan I. Epidemiology of bladder cancer in the arab world: 2019 global burden of disease data. *Asian Pac J Cancer Prev*. 2022;23(9):2907-2919.
3. Li Y, Sun L, Guo X, Mo N, Zhang J, Li C. Frontiers in bladder cancer genomic research. *Front Oncol*. 2021;11:670729.
4. Koutsoukos K, Andrikopoulou A, Dedes N, Zagouri F, Bamias A, Dimopoulos M-A. Clinical perspectives of ERCC1 in bladder cancer. *Int J Mol Sci*. 2020; 21(22):8829.
5. Klatte T, Seitz C, Rink M, et al. ERCC1 as a Prognostic and Predictive Biomarker for Urothelial Carcinoma of the Bladder following Radical Cystectomy. *J Urol*. 2015;194(5):1456-1462.
6. Kawashima A, Takayama H, Tsujimura A. A review of ERCC1 gene in bladder cancer: implications for carcinogenesis and resistance to chemoradiotherapy. *Adv Urol*. 2012:812398.
7. Stoehlmacher J, Park DJ, Zhang W, et al. A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. *Br J Cancer*. 2004;91(2):344-354.
8. Ceppi P, Volante M, Novello S, et al. ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. *Ann Oncol*. 2006;17(12):1818-1825.
9. Dancik GM, Theodorescu D. Pharmacogenomics in bladder cancer. *Urol Oncol*. 2014;32(1):16-22.
10. Kim KH, Do IG, Kim HS, et al. Excision repair cross-complementation group 1 (ERCC1) expression in advanced urothelial carcinoma patients receiving cisplatin-based chemotherapy. *APMIS*. 2010;118(12):941-948.
11. Ozcan MF, Dizdar O, Dincer N, et al. Low ERCC1 expression is associated with prolonged survival in patients with bladder cancer receiving platinum-based neoadjuvant chemotherapy. *Urol Oncol*. 2013;31(8):1709-1715.
12. Ma Q, Lu AY. Pharmacogenetics, pharmacogenomics,and individualized medicine. *Pharmacol Rev*. 2011;63(2):437-459.

13. Bushra MU, Rivu SF, Sifat AE, et al. Genetic polymorphisms of GSTP1, XRCC1, XPC and ERCC1: prediction of clinical outcome of platinum-based chemotherapy in advanced non-small cell lung cancer patients of Bangladesh. *Mol Biol Rep*. 2020;47(9):7073-7082.
14. Roth-Cline M, Nelson RM. Microdosing Studies in Children: A US Regulatory Perspective. *Clin Pharmacol Ther*. 2015;98(3):232-233.
15. Whirl-Carrillo M, Huddart R, Gong L, et al. An Evidence-Based Framework for Evaluating Pharmacogenomics Knowledge for Personalized Medicine. *Clin Pharmacol Ther*. 2021;110(3):563-572.