İSTANBUL UNIVERSITY PRESS

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE
Journal of Advanced Research in Health Sciences / Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2023
DOI: 10.26650/JARHS2023-1273011

GENETIC POLYMORPHISM OF APE1 ASP148GLU IS NOT ASSOCIATED WITH BLADDER CANCER RISK IN A TURKISH POPULATION

TÜRK POPÜLASYONUNDA APE1 ASP148GLU GEN POLİMORFİZMİNİN MESANE KANSERİ İLE İLİŞKİSİ BULUNMAMAKTADIR

Taghi AHMADİ RENDİ[1], Serhat KILINÇ[1], Selçuk ERDEM[1], Enes DEĞİRMENCİ[1], Öner ŞANLI[1], Canan KÜÇÜKGERGİN[1], Semra DOĞRU-ABBASOĞLU[1]

[1] Istanbul University, Institute of Health Sciences, Department of Biochemistry, Istanbul, Turkiye.
[2] Istanbul University, Istanbul Faculty of Medicine, Department of Biochemistry, Istanbul, Turkiye
[3] Istanbul University, Istanbul Faculty of Medicine, Department of Urology, Istanbul, Turkiye

ORCID ID: T.A.R. 0000-0002-2513-6129; S.K. 0009-0002-0483-7599; S.E. 0000-0002-3514-1394; E.D. 0000-0002-5901-0727; Ö.Ş. 0000-0001-5801-6898; C.K. 0000-0002-1797-5889; S.D.A. 0000-0003-3467-9763

Citation/Atıf: Ahmadi Rendi T, Kilinc S, Erdem S, Degirmenci E, Sanli O, Kucukgergin C, et al. Genetic polymorphism in APE1 asp148glu is not associated with bladder cancer risk in a turkish population. Journal of Advanced Research in Health Sciences 2023;6(2):117-121. https://doi.org/10.26650/JARHS2023-1273011

ABSTRACT

Objective: The purpose of this investigative research was to investigate the potential impact of a single nucleotide polymorphism (Asp148Glu) within the APE1 gene on the development of bladder cancer (BCa) and spread, and to investigate the interaction of this polymorphism with cigarette smoking in BCa patients.

Materials and Methods: In a study of 256 participants, consisting of 136 healthy individuals and 120 patients with BCa, the APE1 Asp148Glu polymorphism was evaluated using "polymerase chain reaction restriction-fragment length polymorphism" analysis.

Results: There were no noteworthy difference variations observed in genotype distribution between the group of individuals with BCa and the control group with regards to the APE1 gene polymorphism. Furthermore, the APE1 gene polymorphism exhibited no correlation with the clinicopathological characteristics or smoking habits of individuals with BCa.

Conclusion: Based on our findings, it appears that the APE1 gene polymorphism, which plays a role in the BER pathway, does not appear to be a contributing factor in susceptibility to BCa in the Turkish population. In addition, the smoking habit may not modify BCa risk with respect to genetic variations in the APE1 gene.

Keywords: APE1, polymorphism, base excision repair, bladder cancer

ÖZ

Amaç: Çalışmamızda, APE1 (Asp148Glu) gen polimorfizmi ile mesane kanseri oluşumu ve gelişimi arasındaki ilişkiyi araştırmayı amaçladık. Ayrıca, mesane kanserinde sigara kullanımı ile APE 1 gen polimorfizmi arasındaki ilişkiyi değerlendirmeyi planladık.

Gereç ve yöntem: Çalışmamızda histopatolojik ve klinik açıdan mesane kanseri tanısı konan hastalar (n=120) ve sağlıklı normal kişiler (n=136) yer aldı. Çalışma grubundaki kişilerden elde edilen DNA'lardan Apürinik/Aprimidinik Endonükleaz 1 (APE1) gen polimorfizmi için polimeraz zincir reaksiyonu (PZR), sınırlayıcı enzim parça uzunluğu polimorfizmi (RFLP) teknikleri kullanıldı.

Bulgular: APE1 gen polimorfizminde genotip ve allel sıklığı incelendiğinde mesane kanseri ve kontrol grubu arasında anlamlı farklılık bulunmamaktadır. İlaveten, yüksek grade, ileri evre ve sigara kullanımı bakımından da incelendiğinde anlamlı bir faklılık bulunmamaktadır.

Sonuç: APE 1 gen polimorfizminin mesane kanseri oluşumunda ve gelişiminde bir risk içermediğini ayrıca, sigara kullanımının APE Asp148Glu gen polimorfizminde etkili olmadığını ileri sürebiliriz.

Anahtar Kelimeler: APE1, polimorfizm, baz kesip çıkarma onarımı, mesane kanseri

Corresponding Author/Sorumlu Yazar: Canan KÜÇÜKGERGİN **E-mail:** ckgergin@istanbul.edu.tr

Submitted/Başvuru: 28.08.2022 • **Revision Requested/Revizyon Talebi:** 03.09.2022 • **Last Revision Received/Son Revizyon:** 03.03.2023 • **Accepted/Kabul:** 03.03.2023

INTRODUCTION

Bladder cancer (BCa) is the most commonly diagnosed tumor in the urinary tract and the fourth overall among all male neoplastic diseases (1, 2). Currently, about two million people in the world are struggling with this disease (3). Due to the efforts made in the field of cancer prevention and treatment, the death rate from BCa has decreased significantly over the past few years in many countries, especially in developing countries (4).

In the development of BCa, both environmental factors, including smoking, which is responsible for generating reactive oxygen species (ROS), and genetic factors are involved (5). Free oxygen radicals harm DNA and cause changes in the capacity of DNA repair systems, leading to the appearance of malignant tumors (6). To cope with oxidative DNA damage, the human body employs five distinct repairing DNA mechanisms: direct reversal, double-strand break repair mismatch repair, nucleotide excision repair (NER), and base excision repair (BER), (7, 9). The BER pathway that is in charge of making repairing the damage to DNA induced by oxidation and alkylation, that "apurinic/apyrimidinic endonuclease 1" (APE1) is a main enzyme in BER (7). Additionally, it participates in the redox control of transcription factors in cells and is commonly referred to as redox factor-1. (APE1/Ref-1) (8).

The APE1 gene is situated in the genomic region of chromosome 14q11.2–q12. The Asp148Glu polymorphism, which occurs due to T to G substitution at codon 148 in exon 5 of the APE1 gene, the outcome is the exchange of glutamate for an aspartate amino acid (10). Several reports found that many cancers, involving cancers such as prostate and colorectal cancer while other reports did not find any association with nasopharyngeal cancer or even showed a significant protective effect against BCa have been linked to Glu/Glu genotype of the APE1 Asp148Glu polymorphism as having an elevated risk (11, 13, 14). Therefore, the current research examined the potential impact of the APE1 Asp148Glu gene polymorphism on the development and advancement of BCa within a Turkish population.

MATERIALS and METHODS

Between 2012 and 2022, the Istanbul Faculty of Medicine, Urology Department conducted a study on 120 Turkish patients who had been diagnosed with BCa. A control group consisting of 136 people who were referred to routine medical examinations at the same facility and participants in the study had no prior history of cancer diagnosis of any type. The Ethics Committee, Istanbul Faculty of Medicine, granted ethical approval for the study, and each participant gave informed consent before participating (Date : 23/12/2022, No:23).

BCa was diagnosed histologically with samples received from patients through biopsy or surgery. The tumors were categorized based on their type, grade, and stage. Based on the 2004 WHO grading system for pathological bladder cancers, they were graded as low or high. 2002 TNM classification system was used to classify tumors as either low-stage or high-stage. BCa at a low stage (Ta/T1) is known as superficial BCa (SBC). BCa stages T2 to T4 are known as muscle-invasive BCa (MIBC).

A commercially accessible PCR template preparation kit (Roche Diagnostics, Mannheim, Germany)" was utilized to extract genomic DNA from white blood cells. The polymorphism of the APE1 Asp148Glu (rs 1130409) gene were genotyped using PCR-RFLP. The PCR reactions were carried through in "25 µl of 10 pmol APE1 primer, 0.3 mM dNTP, 2,5 mM $MgCl_{2,}$ 100 ng DNA, x10 PCR buffer (pH value: 8.8), and 1.25 U Taq polymerase (MBI Fermentas)".

The PCR cycling condition for APE1 was initial denaturation at 95 °C for a 2-minute period, 35 cycles of 30 sec at 95 °C, annealing at 52 °C for 45 sec and elongation at 72 °C for 45 sec, and a final extension step of 5 min at 72 °C. Following amplification, PCR products were subjected to restriction digestion using the FspBI enzyme (Thermo Scientific) (Hu et al. 2001). After completion, a 2% agarose gel treated with ethidium bromide was used to conduct gel electrophoresis, and to analyze the final products. Fragment patterns for the APE1 genotypes were AspAsp (164 bp), AspGlu (164,144, and 20 bp), and GluGlu (20 bp).

Statistical analyses

All statistical analyses conducted in this study were performed using SPSS version 21. The collected biochemical parameter data underwent statistical testing using either the Student's t-test for data with equal variances, or the 'Mann-Whitney U test' for data with unequal variances. To determine the difference in genetic distribution between the study group with BCa and the control group, Pearson's chi-square (v2) test was used. We used the Asp/Asp genotype as a reference due to it being the lowest-risk genotype. To assess the correlation between genotypes and tumor grade and stage, Pearson's chi-square test (x^2) was utilized in order to evaluate the total impact of the polymorphism. The statuses of age, sex, BMI, and smoking were determined using a logistic regression, which calculated

Table 1: The demographic and clinical characteristics of the control group and study group diagnosed with bladder cancer (mean±SD)

Parameters	Control group (n=136)	Patient group (n=120)	[a]p value
Age (years)	62.7±6.52	64.2±11.49	0.160
Female/male (%)	19.0/80.1	17.9/82.5	0.630
BMI (kg/m^2)	27.1±2.89	26.5±3.38	0.131
Smoking (%) (never/current)	61.0/39.0	33.3/66.7	0.000
Grade (1/2)		67/53	
Stage (invasive\ superficial)		96/24	

[a]ap: Value from Pearson`s χ^2 test to categorical variables and Mann Whitney-U to continuous variables

Table 2: Distributions of APE1Asp148Glu allele and genotype in controls and bladder cancer patients

	Control group n (%)	Patient group n (%)	p	OR[a](95% CI)
APE Asp148Glu				
AspAsp	93 (68.4)	86 (71.7)		Reference genotype.
AspGlu	40 (29.4)	30 (25.0)	0.767	0.91 (0.48-1.69)
GluGlu	3 (2.2)	4 (3.3)	0.339	1.86 (0.52-6.73)
AspGlu+GluGlu	43 (31.6)	34 (28.0)	0.924	0.97 (0.52-1.78)
Allele				
Asp	226 (83.0)	202 (84.1)		Reference allele.
Glu	46 (17.0)	38 (15.9)	0.742	2.05 (1.47-2.85)

[a]Odds ratios (OR) & 95% CI: Confidence intervals, adjusted for BMI: Age-sex and smoking status

adjusted odds ratios (aOR) and 95% "confidence intervals" (95% CI). To determine the effect size (W) with '2 degrees of freedom (2; 0.05), the NCSS 2000 statistical package (NCSS Inc; Kaysville UT)' was used, taking into account the sample size of the study. Based on these calculations, the study's power was determined to be 83%. The significance degree for the study was set at $p<0.05$, indicating that results with a p-value below this threshold were considered statistically significant.

RESULTS

There were no significant differences found in BMI, age, or sex when comparing the patients with the control group with BCa. However, more smokers were represented in the BCa patients as compared with the controls (p=0.000) (Table 1).

The APE1 Asp148Glu genotypes distribution in the control group was found to be coherent with the 'Hardy Weinberg equilibrium (HWE)' (p=0.587). The distributions of APE1 Asp148Glu genotypes among BCa patients were consistent with the HWE (p=0.496). The effect of APE1 Asp148Glu polymorphism on BCa risk is shown in Table 2. Regarding the controls, the genotype AspAsp was identified in 68.4%, the AspGlu genotype in 29.4%, and the GluGlu genotype in only 2.2% of individuals. The APE1 genotypes were observed in the bladder cancer patients, with 71.7% identified as AspAsp, 25.0% as Asp/Glu, and only 3.3% as GluGlu. There was no noteworthy correlation detected between the APE1 genotype/allele and the risk of BCa (Table 2).

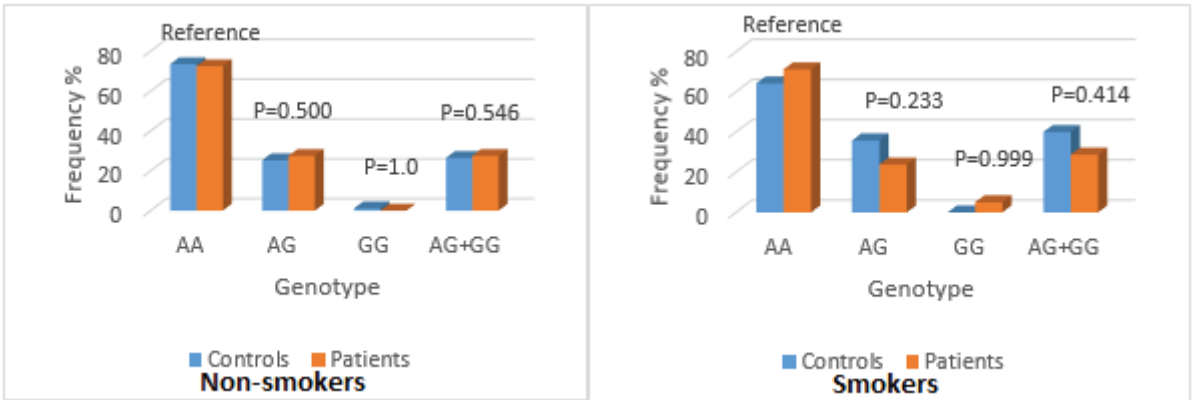

Figure 1: Distributions of APE1 Asp148Glu genotypes related to the smoking status

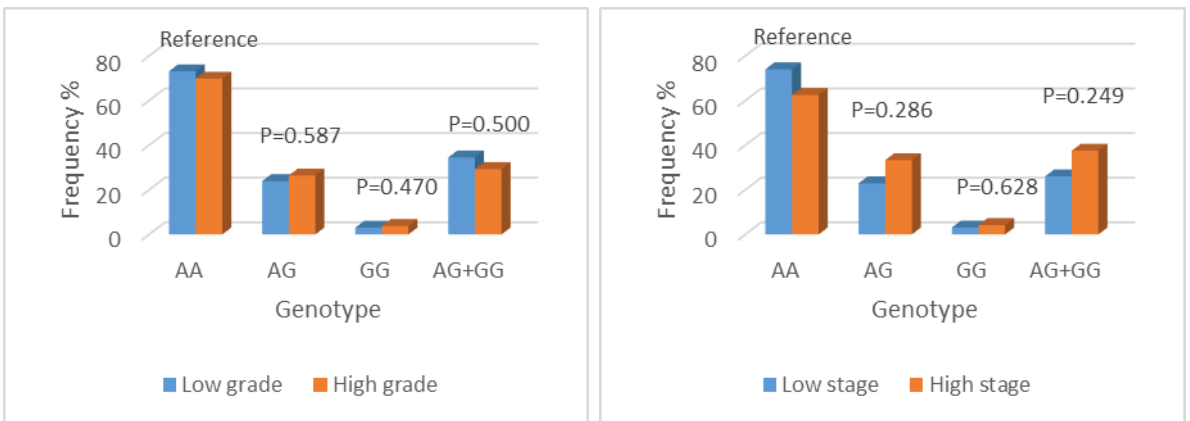

Figure 2: Distributions of APE1Asp148Glu genotypes according to the grade (Low/High) and stage (Low/High) of the disease

The potential dissimilarities in genotype distributions and allele frequencies were investigated between individuals who smoke and those who do not smoke, with respect to their vulnerability to bladder cancer. The APE1 Asp148Glu gene polymorphism was not found to be related within either smokers or non-smokers (Figure 1).

When classified based on BCa grade and stage, the distribution did not show any statistically significant differences in each APE1 genotype, as shown in Figure 2.

DISCUSSION

There are different biological mechanisms in the body as responses to repair DNA damage that preserve the totality of the genome (14). The decrease in capacity for DNA repair efficiency causes changes in the DNA damage-triggered biological response and, as a result, might result in the development of different malignant tumors (15).

Being under the influence of occupational carcinogenic factors is among the other important factors (16). Polycyclic aromatic hydrocarbons, arylamines, nitrosamines, and ROS in cigarettes have been shown to cause significant damage to the DNA molecular structure (17,18).

One of the mechanisms utilized for DNA repair is the BER pathway, which is repairing DNA damage brought on by alkylating agents, ionizing radiation, and oxidation (19). Its ability to function as an endonuclease and phosphodiesterase makes it possible for APE1 to assume a prominent role in repairing apurinic apyrimidinic sites (20).

Polymorphisms in genes responsible for DNA repair that cause changes in the effectiveness of the DNA repair system can be related to the tendency to manifest BCa (22). Of the DNA damage caused by ROS, 8-hydroxyguanine (8-OHG) is particularly mutagenic and results in G-C to T-A transversions during replication by DNA polymerases. (15). The main mechanism responsible for repairing 8-OHG is the short patch BER (15). It has been suggested that polymorphism of DNA repair genes is a risk factor for several cancers, including BCa (1, 23).

Polymorphisms in DNA repair genes cause changes in the amino acid sequence, cause damage to DNA repair capacity, and as a result, cancer appears (24). Various DNA damage is repaired in multiple pathways involving different proteins (25). Although the effect of DNA repair gene polymorphisms on BCa risk has been investigated in many studies, the relationship is not clear yet (1,3,7).

Numerous investigations have been conducted on the relationship between APE1 polymorphism and various cancers. (26). Hu et al. provided further insight into the biological significance of the APE1 codon 148 T-G transversion (Asp-Glu) polymorphism, which has been associated with lymphocyte mitotic delay in healthy individuals and increased sensitivity to ionizing radiation (22). Canbay et al. demonstrated this polymorphism has been linked to a higher incidence of gastric cancer (24). Peng et al. demonstrated APE1 genotypes have a relationship with lung cancer risk (18). The sole recognized common APE1 coding region variant that leads to a non-synonymous change is Asp148Glu. According to Ruchika et al. APE1 148 GG genotype was found to be linked to a protective effect against BCa in a North Indian population (20). Nonetheless, no discernible distinction existed at the allele level (8).

The APE1 Asp148Glu polymorphism did not demonstrate any correlation of significance with the risk of BCa in our study. Our findings are in line with previous research suggesting APE1 Asp 148 Glu polymorphism does not pose a risk for BCa (20, 21).

According to the results of our study data, the finding on gene-environment interaction showed no association between the APE1 Asp 148 Glu genotype and smoking status. Our data are consistent with some previous reports in BCa and prostate cancer (20, 27).

Based on the classification of the studied subjects in terms of disease stage at the time of testing, the APE1 Asp 148 Glu gene polymorphism did not indicate a noticeably higher risk for BCa in more advanced stages. In addition, the risk genotypes for the APE1 Asp 148 Glu polymorphism did not alter tumor grading among BCa patients. Sanyal et al. evaluated that there was no relation between the APE1 Asp 148 Glu gene polymorphism and pathological factors in BCa patients (21). In addition, Liu et al. reported that the APE1 variant allele was not associated with tumor grade and stage in BCa patients (28).

CONCLUSION

According to the results of our investigation, we cannot detect that an association exists between APE1 polymorphism and BCa. The current data from our research's findings demonstrate that APE1 Asp 148 Glu gene polymorphism may not affect the progression of BCa in the Turkish population.

Ethics Committee Approval: This study was approved by Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 23.12.2022, 23).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.D.A., C.K., Ö.Ş.; Data Acquisition- S.E., E.D., T.A.R., S.K.; Data Analysis/Interpretation- T.A.R., S.K.; Drafting Manuscript- T.A.R., E.D., S.K., S.D.A.; Critical Revision of Manuscript- C.K., Ö.Ş., E.D., S.E.; Final Approval and Accountability- C.K., S.D.A., Ö.Ş., E.D., S.K., S.E., T.A.R.; Material and Technical Support- C.K., S.D.A.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was supported by Scientific Research Projects Coordination Unit of Istanbul University. Project number: 38685.

REFERENCES

1. Saginala K, Barsouk A, Aluru JS, Rawla P, Padala SA, Barsouk A. Epidemiology of bladder cancer. Med Sci (Basel) 2020;8(1):15.
2. Wong MC, Fung FDH, Leung C, Cheung WWL, Goggins WB, Ng ACF. The global epidemiology of bladder cancer: a joinpoint regression analysis of its incidence and mortality trends and projection. Sci Rep 2018;8:1129.
3. Antoni SFJ, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol 2017;71(1):96-108.
4. Shah F, Logsdon D, Messmann RA, Fehrenbacher JC, Fishel ML, Kelley MR. Exploiting the Ref-1-APE1 node in cancer signaling and other diseases: from bench to clinic. NPJ Precis Oncol 2017;1:19.
5. Choi S, Shin JH, Lee YR, Joo HK, Song KH, Na YG, et al. Urinary APE1/Ref-1: A potential bladder cancer biomarker. Dis Markers 2016;2016:7276502.
6. Fishel ML, Xia H, McGeown J, McIlwain DW, Elbanna M, Craft AA, et al. Antitumor activity and mechanistic characterization of APE1/Ref-1 inhibitors in bladder cancer. Mol Cancer Ther 2019;18(11):1947-60.
7. Mir SM, Aliarab A, Goodarzi G, Shirzad M, Jafari SM, Qujeq D, et al. Melatonin: A smart molecule in the DNA repair system. Cell Biochem Func 2021;40(1):4-16.
8. Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Influence of XPD and APE1 DNA repair gene polymorphism on bladder cancer susceptibility in North India. Urology 2009;73(3):675-80.
9. Benhamou S, Sarasin A. Variability in nucleotide excision repair and cancer risk: a review. Mutat Res 2000:462(2-3):149-58.
10. Huang HI, Chen CH, Wang SH, Wang LH, Lin YC. Effects of APE1 Asp148Glu polymorphisms on OPMD malignant transformation, and on susceptibility to and overall survival of oral cancer in Taiwan. Head Neck 2019:41(6):1557-64.
11. McIlwain DW, Fishel ML, Boos A, Kelley MR, Jerde TJ. APE1/Ref-1 redox-specific inhibition decreases survivin protein levels and induces cell cycle arrest in prostate cancer cells. Oncotarget 2018;9(13):10962-77.
12. Zhang S, Wang LA, Li Z, Peng Y, Cun YP, et al. APE1 polymorphisms are associated with colorectal cancer susceptibility in Chinese Hans. World J Gastroenterol 2014;20(26):8700-8.
13. Qing Li, Jian-Min Wang, Yu Peng, Shi-Heng Zhang, Tao Ren, Hao Luo, et al. Association of DNA Base-excision Repair XRCC1, OGG1 and APE1 gene polymorphisms with nasopharyngeal carcinoma susceptibility in a Chinese population. Asian Pac J Cancer Prev 2013;14(9):5145-51.
14. Narter KF. Analysis of XRCC3, hOGG1, XPD, XRCC1, XPG and APE1 Gene Polymorphisms in patients with bladder cancer. Istanbul University Institute of Health Sciences, PhD Thesis of Department of Molecular Medicine, 2008.
15. Karahalil B, Aygün Kocabaş N, Özçelik T. DNA repair gene polymorphisms and bladder cancer susceptibility in a Turkish population. Anticancer Res 2006;26(6C):4955-8.
16. Burch JD, Rohan TE, Howe GR, Risch HA, Hill GB, Steele R, Miller AB. Risk of bladder cancer by source and type of tobacco exposure: A case-control study. Int J Cancer 1989;44(4):622-8.
17. Kucukgergin C, Sanli O, Amasyalı AS, Tefik T, Seckin S. Genetic variants of MnSOD and GPX1 and susceptibility to bladder cancer in a Turkish population. Med Oncol 2012;29(3):1928-34.
18. Peng Y, Li Z, Zhang S, Xiong Y, Cun Y, Qian C, et al. Association of DNA base excision repair genes (OGG1, APE1 and XRCC1) polymorphisms with outcome to platinum-based chemotherapy in advanced nonsmall-cell lung cancer patients. Int J Cancer 2014;135(11):2687-97.
19. Ahirwar DK, Marthani A, Mittal RD. IL-8 -251 T>A polymorphism is associated with bladder cancer susceptibility and outcome after BCG immunotherapy in a Northern Indian cohort. Arch Med Res 2010;41(2):97-103.
20. Terry PD, Umbach DM, Taylor JA. APE1 genotype and risk of bladder cancer: Evidence for effect modification by smoking. Int J Cancer 2006;118(12):3170-3.
21. Sanyal S, Festa F, Sakano S, Zhang Z, Steineck G, Norming U, Wijkström H, Larsson P, Kumar R, Hemminki K. Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 2004;25(5):729-34.
22. Hu JJ, Smith TR, Miller MS, Mohrenweiser HW, Golden A, Case LD. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. Carcinogenesis 2001;22(6):917-22.
23. Gu D, Wang M, Zhang Z, Chen J. The DNA repair gene APE1 T1349G polymorphism and cancer risk: a meta-analysis of 27 case-control studies. Mutagenesis 2009;24(6):507-12.
24. Canbay E, Agachan B, Gulluoglu M, Isbir T, Balik E, Yamaner S, et al. Possible associations of APE1 polymorphism with susceptibility and HOGG1 polymorphism with prognosis in gastric cancer. Anticancer Res 2010;30(4):1359-64.
25. Agalliu I, Kwon EM, Salinas CA, Koopmeiners JS, Ostrander EA, Stanford JL. Genetic variation in DNA repair genes and prostate cancer risk: results from a population-based study. Cancer Causes Control 2010;21(2):289-300.
26. Wang M, Qin C, Zhu J, Yuan L, Fu G, Zhang Z, Yin C. Genetic variants of XRCC1, APE1, and ADPRT genes and risk of bladder cancer. DNA Cell Biol 2010;29(6):303-11.
27. Dhillon VS, Yeoh E, Fenech M. DNA repair gene polymorphisms and prostate cancer risk in South Australia results of a pilot study. Urol Oncol 2011;29(6):641-6.
28. Chuan Liu, Qinghua Yin, Lian Li, Ying-zhi, Zhuang Xuyu Zu, Yajie Wang. APE1 Asp148Glu gene polymorphism and bladder cancer risk: a meta-analysis. Mol Biol Rep 2013;40(1):171-6.