Study of Multiple Genotypes in the *XPD* gene for A/C Lys751Gln and Lung Cancer in Samples from Baghdad–Iraq

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Abstract

The chance of treating lung cancer is very low due to late diagnosis of metastasis. Therefore, the study of lung cancer and its related genetic patterns is of great importance in early diagnosis of infected individuals. This study analyzes the replication of A/C Lys751Gln polymorphisms of the XPD gene in the Iraqi population and the risk of its association with lung cancer. The results suggest that the heterozygous genotype Lys/Gln increases the risk of lung cancer, while Lys/Lys reduces at least the risk of lung cancer, WhileGln/Glngenotype does not show any significant increase in the risk of lung cancer. These types of studies help experts in timely diagnosis and use of effective remedial procedures especially in individuals who have patients from family members, relatives and couples in the case of endogamy . We hope that the association between these forms and lung cancer will be more clearly determined with statistical analyses in different populations as well as we suggest using this technique as a diagnostic biomarker in lung cancer patients. This study aimed to assess the effect of polymorphism on genotypes in the *XPD* gene on the susceptibility of lung cancer in Iraqi patients.

Keywords: *XPD, Lung Cancer, Iraq, Polymorphism, RFLP.*

Introduction

Lung cancer is the most common type of cancer and the leading cause of cancer-related deaths worldwide. According to recent statistics, there are more than 25.4 million cases of lung cancer that caused 1.2 million deaths during the period 1990-2016 [1].

In Iraq, lung cancer ranks second among the top ten types of cancer in Iraqi society, with 8.1% of all cancers diagnosed [2]. Lung cancer is a multivariate disease with both internal and external causes, the most common cause of which is smoking, which responsible for 90% of cases in males and 65% of cases in females[3]. This does not mean that non-smokers are safe from the disease, because internal factors as well as other environmental factors play an important role in causing the disease. One of the serious problems in lung cancer is its diagnosis in the late stages (Metastasis), which makes treatment difficult, so analysis of the agents of this type of cancer is of great importance [4].Lung cancer studies have shown a high rate of DNA damage and thus reduced repair capability and increased risk of lung cancer [5]. Studies have shown a defect in the DNA repair system and thus reduced repair capacity and increased risk of lung cancer[6,7].That means that lung cancer is a multi-cause disease (external and internal factors), different variables may affect the DNA repair gene and thus cause damage to the DNA.

One of the most important nuclear repair systems is Nucleotide excision repair (NER) [8],and one of the most effective genes in DNA repair is excision repair cross complementing (ERCC2), also known as the Xerodermapigmintosum group D (*XPD*) [9]. This gene encodes one of the important repair enzymes, and it is believed that the polymorphism in this gene is important in the risk of lung cancer. Studies have confirmed that individuals who have Lys751Gln polymorphism of the *XPD* gene have low repair ability for DNA damage.

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The *XPD* gene is located on the long arm of chromosome 19 (9q13.32) and has a length of approximately 15KD. The protein encoder is a member of the TF11H, a 761 amino acid protein, and contains ATP dependent 5 'to 3' helicase activity of the damaged DNA chain and temporarily reduce the correlation[10, 11]. Studies suggest that polymorphisms in the XPD gene affect the efficiency and ability to repair DNA damage[12]. In Lys751Gln, the Glnamino acid in the codon CAG (C allele)is replaced by the primary Lys amino acid in AAG (A allele) [13]. Thus, polymorphisms in this gene alter the C-terminal terminal structure, which in turn increases the risk of lung cancer by altering the function of the XPD protein[14].

In Iraq there are no previous studies on the model *XPD* Lys751Gln and its relationship to lung cancer by RFLP method, and therefore the study was conducted, and aims to verify its relationship with lung cancer.

Materials and Method

Samples: The current study included 63 Iraqi patients diagnosed with lung cancer ranging in age (30-80) years, including 49 smokers and 14 non-smokers, as well as 24 healthy people as a control group. Blood samples were collected from the visitor patientsat the Teaching Oncology Hospital in the Medical City/ Baghdad for the period from September 2018 to January 2019. After obtaining the moral permission from the hospital and the patients to conduct the study with the help of the consultant doctors, 3 ml of venous blood using by a disposable syringein sterile conditions are kept in tubes containing EDTA at a temperature of -20°C until use.

Genomic DNA Extraction: Blood samples were collected with the aim of extracting DNA using the gSYNC^{TM} DNA extraction Kit from Taiwanese company Genaid.

Genomic DNA: 5 µl of extracted DNA mixed with loading dye and loaded in the wells of 1.5% agarose gel at 75 V for an hour. The results were documented using a UV source at a wavelength of 350 nm [15].Determination of the required segment of the studied genes (target segment) accomplished by using polymerase chain reaction technique,the segment of the XPD gene was 476 bpappeared by using a pair of specific primers according to[16] (Table 1), from Bioneer (Korea) in a lyophilized form. A certain volume of nuclease-free water was added to primer tubes depending on the concentration of the primerrecommended by the leaflet accompanied and the reaction started in the PCR machine according to the program (Table 2) by mixing 5 µl of Pre-mix solution of the kit (Bionner-Korea) with 3 µl of extracted DNA, 2 µlof each primer and 13µl of Nuclease-free distilled water to make the volume up to 25microliters.

Determination of purity and concentration of DNA: DNA samples centrifuged by the centrifuge device to ensure their homogeneity. The estimation was performed using the Nanodrop device, 2 microliters of the extracted DNA placed in the space assigned to the device and the order of measurement is given and recorded ,which ranged from 1.5-1.8 ng per microliters to all samples.

Table 1: Sequence of the pair of pylons used in this study.

Reference	Product size	Sequence (5' - 3')		gene
(16)	476 bp	5'-ATCCTGTCCCTACTGGCCATTC-3'	Forward	XPD
		5'-CCACTAACGTCCAGTGAACTGC-3'	Reverse	

Table 2: Optimal conditions for PCR reaction

No. of cycles	Time	Temperature (C°)	Steps
1	5 minute	94	Initial Denaturation
3	30 second	94	Denaturation
	30 second	62	Annealing
	30 second	72	Extention
1	5 minute	72	Final Extention

Results and Discussions

Analysis of the results of polymerase chain reaction: The molecular size of the PCR product was determined in comparison to (25-2000bp) DNA Molecular weight marker from Bioneer (Korea), 5 µl of the product and 5 DNA ladder on the 2% agarosegel. The voltage was 80V for an hour, and the results of the electrophoresis using the gel imaging unit was photographed under UV light, the molecular weights were measured and the band that magnified by PCR techniqueis in 476 bplocation (Fig. 1).

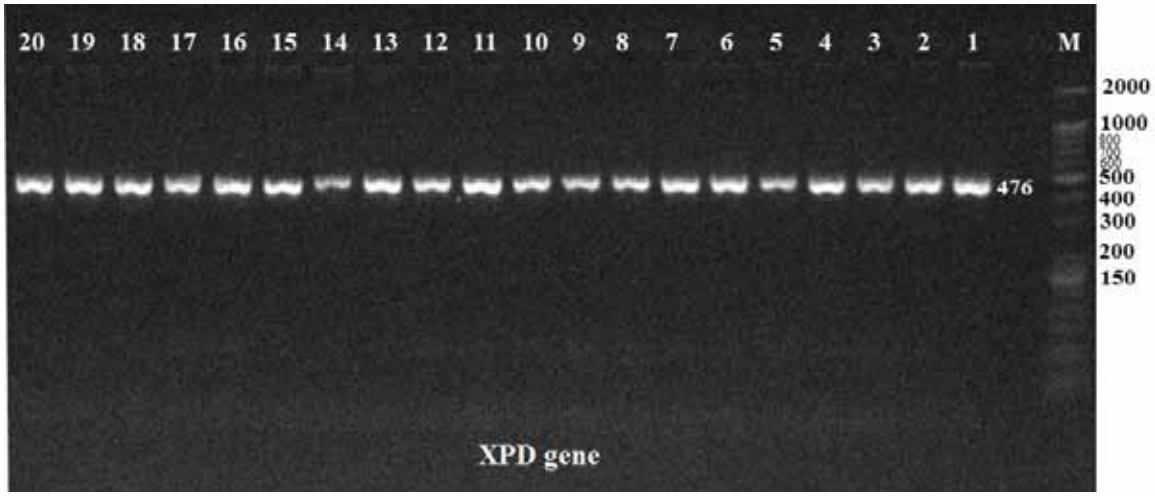

Figure 1: band resulting from amplification of a segment of the *XPD* gene for a number of studied samples (M) Molecular weight marker 25-2000bp from Bioneer/Korea, 1-20are samples studied, size of the amplified fragment is 476 bp.

Enzymatic digestion: 10 microliters of PCR product were taken to be cut by1 µl of *Pst* I restriction enzyme after adding the other additions came with the enzyme kit,the mixture incubate at 37 °C for 1 h and electrophorsed on 3% agarose gel and 80 volts for 1.5 hours.

There is one site to identify the enzyme *Pst* I in allele A, so the allele A is expected to display two bands of DNA in sizes 105, 371 bp, while three bands were expected to appear in the C allele due to the site of the additional cutting. These bands were 63, 105 and 308 bp respectively. Individuals with different alleles of zygote were expected to exhibit a combination of two different alleles (Fig. 2).

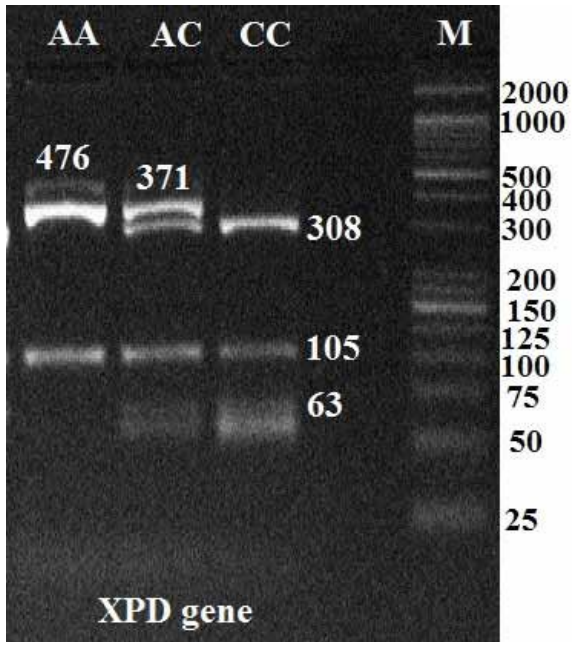

Figure 2: Electrophoresis of PCR-RFLP amplification products using the PstIto analyze Lys751Gln A/C Genotyping, M molecular weight marker 25-2000 base pairs from Bioneer/Korea, CC, AC and AA Genotypes resulting from cutting after amplification of a fragmentof the XPD gene by PCR technology.

Statistical analysis: The statistical analysis system SAS (2012) was used to analyze the various factors in the study. The Chi square law was used to find the range of equilibrium between observed and expected samples. The Hardy–Weinberg equilibrium was used in the population genetics to investigate whether the samples represented the community in real terms. [17].

Distribution of XPD (Lys751Gln) polymorphisms in samples: The genetic polymorphism of a fragment of XPD gene was observed in three hereditary structures (CC, AC, and AA) in patients, and in the absence of XPD genotypes CC in control sample. The homozygous genotype AA recorded the higher percentage in control (53.88%) against that of patients (30.16%)and that of a significant difference (P <0.01). The percentage of the heterozygous genotype AC is (63.49%),and that is higher than in control (46.15%) with a significant difference (P <0.01), while the homozygous genotype GG was recorded in patients (36.35%)and not noticed in control.

The frequency percentage of A allele was (0.62%) in patients and (0.79%) in control, while the frequency percentage of allele C in patients was (0.38%) and in control was (0.21%),and the results of observed values in patients were not consistent with expected values in Hardy-Weinbergequilibrium. In addition, the odds ratio (OR) for the genotype AA was (1.24) and the ratio of (OR) for heterozygous AC genotype was (1.80) and for the homozygous CC was (0.438) (Table3).

Table 3: Distribution and allele frequencies of XPD gene in control and lung cancer groups.

Genotype	Groups		Chi-Square (X^2)	P-Value (O.R.)
	Control No (%)	Lung cancer No (%)		
AA	14 (53.85%)	19 (30.16%)	8.28**	0.0057(1.24)
AC	10 (6.15%)	40 (63.49%)	6.19**	0.0091 (1.80)
CC	0 (0.00%)	4 (6.35%)	2.63 NS	0.072 (0.438)
Total	24	63	-	-
Allele frequency				
A	0.79	0.62	-	-
C	0.21	0.38	-	-

**(P<0.01), NS: Non-Significant

According to statistical analysis, the risk of lung cancer in individuals with polymorphism Lys751Gln (Odd Ratio = 1.80) is greater than that of Lys/Lys genotype, where Gln/Glngenotype is not associated with the risk of lung cancer.

The current study shows that there is a significant difference between patient groups and control in the distribution of AC genotype polymorphism, and C alleles frequency in the Iraqi patient group is more than the control group, so we suggest that this difference could be due to the presence of allele C along with allele A in AC, And molecular interaction between them. These results are consistent with Zhan *etal*.2010[18],who suggested that the AC genotypeof the XPD gene was associated with lung cancer risk and that the C allele in AC genotype of XPD gene was an increased risk factor for developing lung cancer in the meta-analysis studies,as well as consistent with the results of Feng*etal*. 2011[19], who suggested that the AC genotype may contribute to the susceptibility of lung cancer and the high risk of infection with the presence of C allele significantly. It is also consistent with the results of Motovali-Bashi*etal*. 2010 [16], which showed that the proportion of the AC gene was higher in patients compared to the rest of people and that the AA genotype is probably reduces the seriousness. According to a study conducted by Liang *etal*.[20], who mentioned that individuals who have a heterozygous CC genotype, show lung cancer about 2.7 times more than individuals who have an AA genotype.

Conclusion

This study indicates that most of the infections diagnosed occurred for Lys/Gln AC patients. Lys/Lys individuals is likely to reduce its risk with them.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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References

1. Global Burden of Disease Cancer Collaboration. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability adjusted life-years for 32 cancer groups, 1900 to 2016: a systematic analysis for the global burden of disease study. JAMA Oncol. 2018; 4(11): 1553-1568.
2. Iraqi Cancer registry center publications, (2015). Ministry of Health, Iraqi Cancer Board/Baghda.
3. Xie, A.; Croce, B.; Tian, D. H. Smoking and lung cancer. Ann Cardiothoac Surg. 2014; 3(2):221-228.
4. Shanbeh, Z.; Vidar, S. Single nucleotide polymorphisms susceptibility, prognostic and therapeutic markers of non small cell lung cancer. Lung Cancer. 2012; 3: 1-14.
5. Wu, X.; Roth, J. A.; Zhao, H. Spitz M.R., et al. Cell cycle checkpoints, DNA damage/repair, and lung cancer risk. Cancer Res. 2005; 65: 349-357.
6. Wei, Q.; Cheng, L.; Hong, W.K.; Spitz M. R. Reduced DNA repair capacity in lung cancer patients. Cancer Res., 1996; 56(18): 4103-4107.
7. Spitze M. R.; Wei, Q.; Dong, Q.; Amos C. I.; Wu, X. Genetic susceptibility to lung cancer: the role of DNA damage and repair. Cancer Epidemiol Biomarkers Prev., 2003; 12(8): 689-698.
8. De Boer, J.; Hoeijmakers, J. H. Nucleotide excision repair and human syndromes. Carcinogenesis. 2000; 21(3): 453-460
9. Banescu, C.; Trifa, A.P.; Demian, Set al. Polymorphism of XRCC3, and XPD genes and risk of chronic myeloid leukemia. BioMed Res Int 2014; 2014: 1D213790.
10. Leibeling, D.; Lapse, P.; Emmert S. Nucleotide excision repair and cancer. J MolHistol. 2006; 37(5-7): 225-238.
11. Behamou, S.; Sarasin, A. ERCC2/XPD gene polymorphisms and lung cancer: A HuGE review. Am J Epidemiol. 2005; 16(1): 1-14.
12. Spitz, M. R.; WY, X.; Wang, Y.; Wang, L.E.; Shete, S.; Amos, C.I., et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res. 2001; 61(4): 1354-1357.
13. Butkiewicz, D.; Rusin, M.; Enewold, L.; Sheilds, P. G.; Chorazy, M.; Harris, C. C. Genetic polymorphisms in DNA repair genes and risk of lung cancer. Carcinogenesis. 2001; 22(4): 593-597.
14. Monaco, R.; Rosal, R.; Dolan, M. R.; Freyer, G.; Brandt-rauf P. W. Conformational effects of a common codon 751 polymorphism on c-terminal domain of xerodermapigmentosum D protein. J Carcinog. 2009; 8: 12.
15. Maniatis, T.; Fritsch, E. F. &Sambrook. (2001). In vitro applications of DNA by the polymerase chain reaction, in molecular cloning: A laboratory manual. 2nd ed., Cold Spring Harbor lab.
16. Motovali-Bashi, M.; Rezaei, H.; Dehghanian, F.; Rezaei, H. Association between XPD (Lys751Gln) polymorphism and lung cancer risk: a population-based study in Iran. Cell J. 2014; 16(3): 309-314.
17. SAS. (2012). Statistical analysis system, users guides. Statistical Version 9.1th ed. SAS. Inst. Inc. Cary. N. C. USA.
18. Zhan, P.; Wang, Q.; Wei, S.Z.; Wang, J.; Qian, Q.; Yu, L.K.; Song, Y. ERCC2/XPD Lys751Gln and Asp312Asn gene polymorphism and lung cancer risk: a meta-analysis involving 22 case-control studies. J. ThoracOncol. 2010; 5(9): 1337-1345.
19. Feng, Z.; Ni, Y.; Dong, W.; Shen, H. and Du, J. Association of ERCC2/XPD polymorphisms and interaction with tobacco smoking in lung cancer susceptibility: a systemic review and meta-analysis. Mol. Biol. Rep. 2011; 39(1): 57-69.
20. Liang, G.; Xing. D.; Miao, X.; Tan, W.; Yu, C.; Lu, W., et al. Sequence variations in DNA repair gene XPD and risk of lung cancer in a Chinese population. Int J Cancer. 2003; 105(5): 669-673.