Original Article

Association between XRCC-1 Arg194Trp Polymorphism with Lung Cancer in Iraqi Patients

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Abstract

Background: Preface, DNA repair is the most efficient protective approach against DNA damage. Because the integrity of the genome is vital and crucial, the process of repairing DNA induced by carcinogens is critical. The ability to repair DNA damage may be impacted by specific genetic variants in DNA-repair genes, which may also be a risk factor for the development of cancer. The X-ray repair cross complementing-1 (XRCC-1) gene product has been linked to base-excision and single-strand repair mechanisms. **Objective:** The goal of this study was to examine the relation of the XRCC-1 gene with lung cancer patients. **Materials and Methods:** polymarease chain reaction- restriction fragment length polymorphism (PCR-RFLP) analysis was used to compare 40 lung cancer patients along with 40 sample controls. Samples were collected at Al-Amal National Hospital for cancer management, ages ranging from 40 to 70 years. Statistics were carried out by using the SPSS Software program Version 20.0. **Results:** The results show that the XRCC-1 (Arg194Trp) gene carried seven exons, which were shown to have different frequencies in patients and controls based on three genotypes (Arg/Trp, Arg/Arg, and Trp/Trp). Hardy–Weinberg equilibrium with chi-square for the patient (7.949) and control group (8.236). Also, the Arg/Trp genotype was present at a significantly high frequency in patients (77.5%), and the odds ratio was (14.94, confidence interval [CI] = 4.99–44.76 to CI = 0.642), whereas the Trp/Trp genotype frequency increased in the control group (72.5%). And the odds ratio for this relationship was (0.3, P = 0.001, and CI = 0.01–0.11). **Conclusion:** This study's findings also suggest that the Arg/Trp genotype may be linked to lung cancer in this particular group.

Keywords: DNA repair, genotype, lung cancer, PCR-RFLP, polymorphism, XRCC-1 Arg194Trp

Introduction

Lung cancer is one of the first positions on lethal diseases lists, with a very low survival rate.[1] Lung cancer incidence has steadily increased in recent years.[2] Its danger has been related to a variety of environmental and genetic causes. Toxins in the environment and cigarette smoking are thought to induce lung cancer.[3] Despite the fact that cigarette smoking is still the leading driver of lung cancer but it does not explain the epidemic features of lung cancer among nonsmokers.[4] Variations in the development of lung cancer are thought to be mostly determined by genetic susceptibility to environmental or occupational diseases.[5]

Furthermore, genetic polymorphisms in DNA repair genes have been linked to genomic instability and increased risk of genomic damage.[6] DNA repair is widely recognized for its role in genomic integrity maintenance, in which it repairs DNA damage caused by both exogenous and endogenous cancers. DNA repair gene polymorphism is expected to alter the ability to repair DNA damage, leading to changes in base excision repair (BER) capacity, and may be a risk factor for a variety of malignancies.[7] Repairing X-ray, X-ray repair cross complementing-1 (XRCC-1) is a gene that codes for a single strand break repair protein. As a result, XRCC-1 interacts with other

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DNA repair proteins; it serves a critical function to interact with the BER pathway. It is possible that XRCC-1 has anything to do with lung cancer responsiveness to therapy and overall survival.[8,9] A previous study has linked several genetic variants, including XRCC-1 gene changes that increase the risk of cancer.[10,11] The goal of this study was to examine the relation of the XRCC-1 gene with lung cancer patients.

Materials and Methods

DNA extraction

This study comprised 40 lung cancer patients and 40 healthy (control), ages ranging from 40 to 70 years. The samples were collected from blood at Al-Amal National Hospital in Baghdad, Iraq. The duration of sample collection was between September 2021 and February 2022. DNA was extracted according to the instructions of the manufacturer using a commercially available kit (ZYMO). DNA concentration was carried out by Promega kit (Promega).

DNA amplification

Restriction enzymes used in the RFLP technique were MspI (Takara Bio, Shiga, Japan), which was used to digest PCR product of 491 bp and was digested at 37C° for 3 h. Table 1 shows Primer sequences used to amplify the XRCC-1 gene by PCR, in addition to the restriction enzyme.

After identifying the ideal conditions for (initial denaturation and annealing), a volume of 25 L from Master Mix PCR (Promega, Wisconsin, United States) is 5 L, 10 pmol. Of each primer, 1.5 L DNA and, finally, the nuclease-free water is added to the total volume. Table 2 depicts a PCR program. Visualization with a UV transilluminator was accomplished after staining with a red-safe stain.

The restriction digestion procedure followed industry standards. The PCR products were divided into ten microliters, and each one was digested with (0.5 L) of MspI. 18 mL of Q water and 2 mL of 10× buffer made up the reaction mixture.

Statistical analysis

SPSS Statistics for Windows, Version 23.0 (IBM SPSS Statistics for Windows, Version 20.0) was used to perform analysis on the data (Armonk, New York: IBM Corporation) in addition to Microsoft Excel 2019.

Ethical approval

All investigations were carried out in compliance with the Ministry of Health ethics committee according to document number 72240 on March 13, 2021, to get this approval.

Results

RFLP techniques were used for genotyping of XRCC-1 Arg194Trp (exon 7), the bands detected by gel electrophoresis as shown in Figure 1. The restriction enzyme MspI was used to digest the PCR products of these samples to identify the genotype of Arg/Arg, which is represented by 21 + 292 bp, Arg/Trp by 21 + 292 + 313 bp and Trp/Trp by 313 bp.

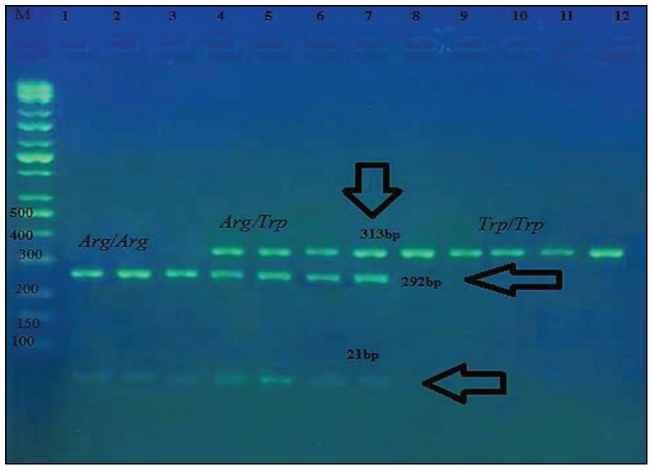

Figure 1: The XRCC-1 gene was electrophoresed on an agarose gel. Following staining with red stain, bands separated by 2% agarose gel electrophoresis at 5 V/cm, 1× TBE buffer for 2 h, then visualized by UV gel documentation. Lane M: refer to DNA marker (100 bp range), Lane: 1, 8, 9, 10, 11, 12 for the control group, Lane: 2, 3, 4, 5, 6, 7 for patients' group

Table 1: Primer used for RFLP-PCR reactions

XRCC-1 gene	Primer sequences (5′-3′)	Restriction enzyme	Size	References
Forward	GCCCCGTCCCAGGTA	MspI (Japan, Takara) digested at 37°C for 3 h	491 bp	[12]
Reverse	AGCCCCAAGACCCTTTCACT			

Table 2: PCR programing

Step	Temperature (C°)	Time	Cycle number
Initial denaturation	94	3 min	1
Denaturation	94	45 s	30
Annealing	57	45 s	
Extension	72	45 s	
Final extension	72	7 min	1

Table 3: XRCC-1 Arg149Trp gene frequency in both patients and control group according to Hardy–Weinberg equilibrium

Groups			Genotypes			HWE $P > 0.05$	Alleles	
			Trp/Trp	*Arg/Trp*	*Arg/Arg*		*Trp*	*Arg*
Control	Observed	No.	29	7	4	8.236	66	14
		%	72.5	17.5	10		82.5	17.5
	Expected	No.	26.22	12.56	1.22			
		%	67.05	29.89	3.06			
Patients	Observed	No.	3	31	6	7.949	35	45
		%	7.5	77.5	15		43.75	56.25
	Expected	No.	7.65	21.68	10.67			
		%	19.12	49.2	31.68			

Table 4: Allele frequency, genotyping and statistical analysis of patients and control group

Genotypes	Control %	Patient %	*P*-value	OR	Etiological/prevention	95% CI
Arg/Trp	7 (17.5)	31 (77.5)	0.001	0.03	37	0.01–0.11
Trp/Trp	29 (72.5)	3 (7.5)	0.001	14.94	0.07	4.99–44.76
Arg/Arg	4 (10)	6 (15)	0.248	2.25	0.44	0.63–8.05
Allele frequency (%)						
Allele	Control	Patients	*P*-value	OR	Prevention	95% CI
Trp	66	35	0.001	0.16	6.06	0.08–0.34
Arg	14	45				

OR = odds ratio, 95% CI = 95% confidential interval

XRCC-1 (Arg194Trp) gene carried seven exons, which were shown to have different frequencies in patients and controls based on three genotypes (Arg/Trp, Arg/Arg, and Trp/Trp). Hardy–Weinberg equilibrium is indicated in Table 3 with chi-square for the patient (7.949) and control group (8.236).

Table 4 shows the genotype and allele frequencies of patients and control groups; the results show that the frequency of the Trp/Trp allele was higher in the control group than in the patients (72.5%, 7.5%, respectively), while the odds ratio was (14.94, $P = 0.0001$ and CI = 4.99–44.76) with prevention factor of (0.07). While the Arg/Trp allele genotyping was more common in patients than in the control groups (77.5%, 17.5%, respectively), while the odds ratio for this unfavorable relationship was (0.03, CI = 0.01 to 0.11, $P = 0.001$) with etiological factor (37). The allele genotyping of Arg/Arg was found no significant between the control and patient groups ($P = 0.248$) with greater frequency than control (15%, 10%, respectively).

Discussion

DNA repair mechanisms are vital for genomic integrity and carcinogenesis prevention, with XRCC-1 being the most important component of the BER pathway.[13] The result indicated that the Arg/Trp genotype was much more common in Iraqi patients with lung cancer than in control carrying (Trp/Trp), which was consistent with research done on the North Indian population by Pachouri *et al*.[14] Numerous studies in molecular epidemiology have investigated the impact of XRCC-1 gene polymorphisms on lung cancer.[15-17] The XRCC-1 gene has three well-studied single nucleotide polymorphisms, all of which are common. Certain research has investigated XRCC-1 gene polymorphisms with the chance of getting lung cancer in patients,[18,19] which is consistent with our findings. According to David-Babes and London,[20] having the codon 194 variant allele (Trp) reduced the incidence of lung cancer in both black and white people. In China, the XRCC-1 codon 149 Trp/Trp genotype and lung cancer risk were reported to be adversely linked 20. Butkiewicz *et al.*[21,22] discovered there was no relationship between XRCC-1 polymorphism and lung cancer risk in a study comprising 96 patients with non-small cell lung cancer and 96 healthy controls in Poland, which contradicts our findings. Previous research on the impact of XRCC-1 Arg149Trp polymorphism on lung cancer risk discovered that homozygotes Trp/Trp variant genotypes may increase lung cancer risk in the general population, particularly among Asians; conversely, heterozygotes Arg/Trp variant genotypes may reduce lung cancer risk in the general population, particularly among whites' peoples.[13,23] While Zhang *et al.*[24], found no link between Arg194Trp polymorphism and incidences of lung cancer in Asians or Caucasians; this contradicts our findings. In contrast another show, people who drank alcohol and had the mutant Arg194Trp allele appeared to have a decreased chance of developing lung cancer than people who had the homozygous wild-type genotype. XRCC-1 polymorphisms appear to affect lung cancer risk and may alter risk related to environmental exposures.[25,26]

Conclusion

In a nutshell, XRCC-1 variants in Iraqi patients and controls were linked to lung cancer. The findings imply that the XRCC-1 (Arg194Trp) gene may be a lung cancer risk genotype in Iraqi patients.

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Conflicts of interest

There are no conflicts of interest.

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