ORIGINAL ARTICLE

# ASSOCIATION BETWEEN APE1 GENE AND LUNG CANCER IN IRAQI POPULATION

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## ABSTRACT

**The aim:** To find association between ape1 gene and lung cancer in Iraqi population.

**Materials and methods:** This study included forty patients with lung cancer and forty people of control group, ranging in age from 40 to 65 years old.

**Results:** The results of (Asp/Glu) genotype showed a significant (p=<0.01) higher frequency in patients than in control group carrying the (Asp/Asp).

**Conclusions:** APE1 Asp148Glu polymorphism may bear a risk for development of the lung cancer in Iraqi patients, and the Asp/Glu genotype contributed to more often predisposal of the disease by playing an important role as increased activity of gene as a result of APE1 Asp148Glu (rs1130409) polymorphism, while Asp/Asp genotype may have a protective action against this disease.

**KEY WORDS:** APE1, Polymorphism, Lung cancer

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## INTRODUCTION

The most prevalent type of cancer is lung cancer form that remains the leading cause of cancer-related death globally [1]. The most frequent kind of lung cancer is non-small cell lung cancer (NSCLC), which is 85 percent of all cases. Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the two histological kinds [2]. The mechanism of lung cancer development is unknown. Despite the fact that smoking is the single most important agent in lung cancer, host factors such as genetic polymorphism may lead to development of lung cancer that makes it interesting for research of all agents of carcinogenesis [3]. Defects in cell cycle checkpoints and DNA damage/repair capabilities may play a role in the increased risk of lung cancer [4]. The DNA repair gene system is essential for preventing gene mutations caused by tobacco smoking. Single nucleotide polymorphisms (SNPs) in DNA repair genes have recently been discovered to be the underlying biological basis of individual diversity in DNA repair capacity [5]. The APE1 is a key enzyme in base excision repair pathway, and this enzyme is responsible for repairing DNA damage induced by oxidation/alkylation and protecting cells from endogenous and external agents [6].

## THE AIM

To find association between ape1 gene and lung cancer in Iraqi population.

## MATERIALS AND METHODS

This study included forty patients with lung cancer and forty people of control group, ranging in age from 40 to 65 years old. Samples were gathered at Alamal National Hospital for Cancer Management in Baghdad, Iraq, among August 2020 and January 2021 for the study. A commercially available kit was used to extract the DNA as indicated by the manufacturer (ZYMO, USA). The DNA concentration was determined using a Quantus (Promega, USA), and samples were stored at -20 ° C until needed (RFLP-PCR). The APE 1 was amplified utilizing primers (IDT, USA) as in figure 1.

The thermal cycling program were as follow, enzyme activation 95 °C for 7 min, followed by 40 cycles of two steps: the first one was denaturation 95 °C for 45 sec and second step of annealing and florescence screening for 20 sec (52 °C) and extension for 45 sec.

## RESULTS

The genotyping from Asp148Glu were done by using RFLP technique and bands have been visualized by gel electrophoresis as shown in figure 2. PCR product of these sample were digested using the restriction enzyme (FspBI). The PCR products that have been treated with restriction enzyme to detect the genotype Asp/Asp represented as 164bp, and genotype Asp/Glu represented by 164/144/20, and Glu/Glu represented by 144/20.

The SNP rs1130409, which is located in the fourth exon of the APE1 gene, showed varied frequencies in both patients and control regarding to the three genotype ASP/ASP, ASP/Glu and Glu/Glu. The results from frequencies rejected Hardy-Weinberg equilibrium as shown in table I, as the chi-square for patients equal to (8.949) and control was equal to (9.236).

| Primer name | *primer sequence (5'-3')* | PCR Product | Restriction enzyme |
|---|---|---|---|
| F | 5'- CTGTTTCATTTCTATAGGCTA - 3' | 164bp | FspBI(Thermo Scientific, USA) at 37°C for 6 h |
| R | 5'- AGGAACTTGCGAAAGGCTTC -3' | | |

**Fig. 1.** Primers used for PCR reactions, and restriction enzyme [7]

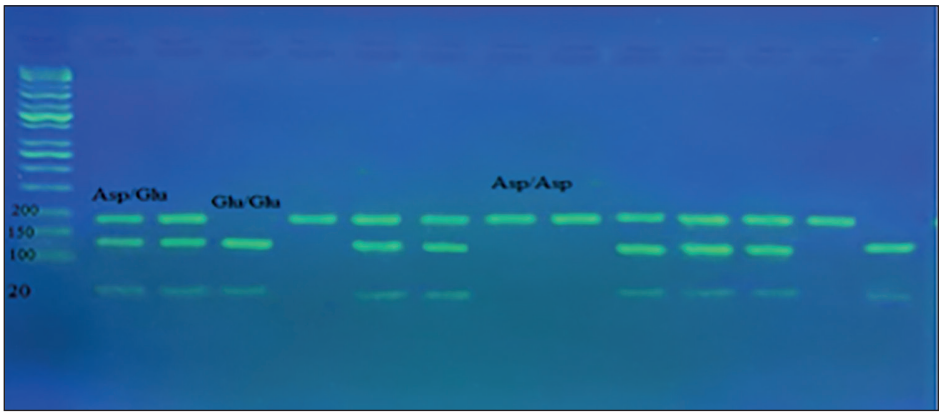


**Fig. 2.** Agarose gel electrophoresis for APE1 gene (164bp) . The picture obtained after electrophoresis on 2% agarose, 5V/cm, 1X TBE. Lane: ladder (M: 100bp, Asp/Asp=164bp, Asp/Glu=164+144+20bp, Glu/Glu=144+20bp.)

**Table I.** Observed and expected numbers and frequencies of rs1130409 in both patients and control group, compared to Hardy Weinberg equilibrium

| | Groups ASP/ASP ASP/Glu | | Genotypes | | | HWE p>0.05 Glu | Alleles | |
|---|---|---|---|---|---|---|---|---|
| | | | Glu/ Glu | | ASP | | | |
| Control | Observed | No. | 30 | 6 | 4 | 9.236 | 66 | 14 |
| | | % | 75 | 15 | 10 | | 82.5 | 17.5 |
| | Expected | No. | 27.22 | 11.55 | 1.22 | | | |
| | | % | 68.05 | 28.87 | 3.06 | | | |
| Patients | Observed | No. | 3 | 29 | 8 | 8.949 | 35 | 45 |
| | | % | 7.5 | 72.5 | 20 | | 43.75 | 56.25 |
| | Expected | No. | 7.65 | 19.68 | 12.65 | | | |
| | | % | 19.12 | 49.2 | 31.62 | | | |

**Table II.** Genotypes frequencies and statistical differences between patients and control group

| Genotype | Control (%) | Patients (%) | P-value | Odds | etiological/ prevention | 95% CI |
|---|---|---|---|---|---|---|
| ASP/ASP | 30 (75) | 3 (7.5) | 0.001 | 0.03 | 37 | 0.01 to 0.11 |
| ASP/Glu | 6 (15) | 29 (72.5) | 0.001 | 14.94 | 0.07 | 4.99 to 44.76 |
| Glu/ Glu | 4 (10) | 8 (20) | 0.348 | 2.25 | 0.44 | 0.63 to 8.05 |
| **Allele frequency (%)** | | | | | | |
| **Allele** | **Control** | **Patients** | **P-value** | **Odds Ratio** | **etiological/ prevention** | **95% CI** |
| Asp | 66 | 35 | 0.001 | 0.16 | 6.06 | 0.08 to 0.34 |
| Glu | 14 | 45 | | | | |

The genotypes and alleles frequencies of both patients and control groups, combined with statistical analysis, are seen in table II. The results showed genotype ASP/ASP frequency increased in control group more than in

40 patients (75% and 7.5%, respectively) the odd ratio for this relation was (0.03, p= 0.001 and C.I. = 0.01 to 0.11) with prevention factor equal to (37). While the ASP/Glu genotype showed significant high frequency in patients than in control group (72.5% and 15%, respectively) the odds ratio for this strong negative relation was (14.94, C.I.= 4.99 to 44.76, p= 0.642) with etiological factor equal to (0.07). on another hand, Glu/Glu genotype showed non-significant (p=0.348) higher frequency in patients than in control group (20% and 10%, respectively).

## DISCUSSION

DNA damage can be caused by endogenous and/or external exposure, such as exposure to toxins in cigarette smoke [8]. DNA repair systems serve a critical role in ensuring the genome's integrity. This defensive system's deficiencies are hypothesized to have a role in the development of cancer. DNA repair capacity has been linked to DNA repair ability may be influenced by genetic variety in DNA repair genes, which may increase cancer risk. As a result, DNA repair genetic polymorphisms may influence cancer vulnerability [9]. Tobacco smoking induces oxidative damage by releasing reactive oxygen species, and BER genes help to repair DNA damage caused by oxidation, deamination, and ring fragmentation [10]. In the BER pathway, APE1 is the rate-limiting enzyme [11]. Despite the fact that the APE1 Asp148Glu polymorphism has no effect on endonuclease activity [12], people who have the Glu allele may be more sensitive to ionizing radiation [13]. Many DNA repair gene polymorphisms have been investigated in the context of lung cancer and/or DNA repair ability. According to several studies, there is no relationship among the APE1 Asp148Glu polymorphism and the risk of lung cancer [14,15]; however this differs from our study and other studies that discovered a strong link [16], and which agree with our study. We found a substantial link between the Asp148Glu heterozygous genotype and lung cancer risk in Iraqi patients, which agrees with the findings of De Ruyck et al. [16], who found a link between the Asp/Glu genotype and lung cancer risk in Caucasians. In male smokers, Misra et al. [11] found a Glu allele frequency from 0.52 for APE1 codon 148. A Chinese study found a link among the APE1 148 Glu allele and an elevated danger of lung cancer among heavily smoking male [17].

## CONCLUSIONS

APE1 Asp148Glu polymorphism may bear a risk for development of the lung cancer in Iraqi patients, and the Asp/Glu genotype contributed to more often predisposal of the disease by playing an important role as increased activity of gene as a result of APE1 Asp148Glu (rs1130409) polymorphism, while Asp/Asp genotype may have a protective action against this disease.

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**Contributionship:**
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**Conflict of interest:**
*The Author declare no conflict of interest.*

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