RESEARCH ARTICLE

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Association of the Manganese Superoxide Dismutase (Mn-SOD) Gene C47T Polymorphism with Lung Cancer: A Case-Control Study

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Abstract

Objective: The objective of this study was to determine the association between manganese superoxide dismutase (*Mn-SOD*) gene C47 T polymorphism and the risk of malignant lung cancer in Iraqi smokers. **Methods:** Blood samples were obtained from 260 lung cancer patients (88 females and 172 males) and 295 healthy individuals (91 females and 204 males). DNA was extracted from blood samples and the *SOD2* gene was amplified using specific primers. The nucleotide sequences of the *SOD2* gene were analyzed by using BLAST server at National Center for Biotechnology Information (NCBI) and the Raptorx app. **Results:** TT, CT, and CC genotypes concentrations were 48.1%, 33.2%, and 18.7%, respectively, in the control group. The concentrations of TT, CT, and CC genotypes were 43.5%, 31.5%, and 25%, respectively, in the case group. There were no statistical differences between cases and controls in terms of genotype frequency of SOD2C47T polymorphism. We observed that SOD2C47T polymorphism CT genotype did not increase the risk of lung cancer development compared to those with TT genotype (OR= 0.951, 95% CI = 0.648-1.396; P = 0.798). In addition, it was observed that CC genotype did not increase the risk of lung cancer development in comparison with TT genotype (OR=0.673, 95% Cl=0.435-1.041: P=0.075).**Conclusion:** These results indicated that there was no association between SOD2C47T polymorphism and the risk of lung cancer development in Iraqi smokers.

Keywords: Mn2- lung cancer- polymorphism

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Introduction

Lung cancer is a significant cause of cancer-related death in developed countries, leading to extremely low overall survival rate . Cigarette smoking is a proven risk factor for lung cancer although the family clustering of the disease and segregation analyzes have suggested a possible role for genetic susceptibility in the development of lung cancer (Kiyohara et al., 2002).

In Iraq, lung cancer is one of the top five malignant tumors and a leading cause of cancer-related mortality, especially in southern Iraq as a result of the first and second Gulf War. In this war, explosives containing uranium isotope 238were used, causing DNA mutations. The precise number of cases with lung cancer is not known . On the other hand, it is not known whether the cause of these cancers is the heavy use of cigarettes or pollution caused by the use of nuclear weapons in the Iraq war (Omran et al., 2016).

Cigarette smoke contains several thousand chemicals known as chemical-modified DNA, resulting in DNA mutations in . The compounds that makeup tobacco are carcinogens. Such compounds are capable of binding to DNA and shaping DNA that can cause mutations and carcinogenesis initiation. One of the host factors that can affect the risk of lung cancer is the repair of DNA damage caused by these carcinogens (Chikako et al., 2010; Herbst et al., 2008; Livneh, 2001).

It is understood that reactive oxygen species (ROS) cause DNA damage, which consequently results in genetic mutations that induce mutagenic activity and tumorigenicity. ROS increase the rate of mutation in cells and promote oncogenic transformation. DNA damage, including oxidized bases, DNA adduct formation, and DNA double-strand breaks, causes genomic instability. Such effects of ROS may be counteracted by the antioxidant action of non-enzymatic antioxidants or antioxidant enzymes (Kang, 2015; Janicka et al., 2013; Zhang et al., 2011).

The association between SOD2 polymorphism and cancer risks was studied in many previous studies, but the results are still controversial. After Crawford et al.' s report on the association between single nucleotide polymorphisms of antioxidant enzymes and cancer risk

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in their meta-analysis in 2012 (Crawford et al., 2012), the relationship between SOD2 polymorphism and the risk of other cancers was reported in more studies.

Materials and Methods

Patients and clinical sampling

The research was carried out on 260 patients with lung cancer (172 males and 88 females), all of whom were diagnosed with lung cancer between April 2018 and February 2019 at the Cancer Oncology Center at Diwaniyah Teaching Hospital in Diwaniyah and continued to smoke more than 20 cigarettes a day after their disease. . A control group comprised of 295 individuals (204 females and 91 males) was considered representing a population without cancer. Everyone has been thoroughly tested and smoke their medical history clinical and regular laboratory tests with cellular analysis or pathological anatomy of tumor biopsies are performed to diagnose lung cancer. We obtained 2 ml of peripheral blood keeping a tube containing ethylenediaminetetraactic acid (EDTA). Before further analysis, the specimens were deposited at - 20 C based on previous studies (Hoidy et al., 2019; Jabir and Hoidy, 2018).

Measurement of DNA concentration and purity

Nanodrop (Quawell / Hong Kong) was used to test the extracted DNA concentration and purity. For this purpose, 2 mL of DNA was quantitated on the NanoDrop lens and measured at a wavelength of 260/280-nm. The result appeared on the NanoDrop which attached to laptop screen. After each sampling, the NanoDrop lens were washed with distilled water and cotton swab.

The researcher then developed primers for genotyping of *SOD2C47T C / T* gene fragments using NCBI and Sigma Aldrich's multiple priming design software. (Table 1). The target fragment containing SOD2C47T polymorphism was amplified. SOD2C47T polymorphism fragments were amplified in a reaction mixture of 20 mL containing 2 µl of genomic DNA template, 1 µl of each reference, 13 µl H_2O, and 5 µl of PCR master mixture (Pioneer, southern koria). The PCR product was then separated on 2% agarose gel at 50 V for 45 minutes in 0.5X tris/borate/EDTA buffer by taking 5 mL from each sample. Agarose gels were treated for 20 to 30 minutes with a safe stain 0.5 mg / mL.

Statistical analysis

The frequencies of genotype and allele carriers were specified as the percentage of people carrying the genotype and alleles. The X^2 and P values < 0.05, the odds ratio and their 95 percent SPSS 23.0 confidence interval measures for Windows (IBM Corp, Armonk, NY) were used to compare the frequency of discrete variables between breast cancer patients and controls.

Results

A total of 555 individuals were examined in this study (295 healthy individuals and 260 patients with lung cancer. Table 2 summarizes the demographic characteristics of the participants. Patients and controls were matched in terms of gender, age, weight, height, and BMI. The means of age, weight, height, and BMI were 62.26±2.5, 70.19±7.1, 171.66±6.3 and 22.56±5.2, respectively, in the control group. The means of age, weight, height, and BMI were 58.17±9.2, 75.46±3.7, 174.92±4.1, and 24.66±2.8, respectively, in the case group (Table 2).

Figure 1 shows gel electrophoresis of DNA extraction for patients and controls. The extension from these archival samples of the target sequence of SOD2C47T Polymorphism resulted in 320 bp. The amplified fragment, which produced a single band of the desired product with a molecular weight of genes, was found to be sharp in agarose gel using a gel electrophoresis technique and loaded with a 50 to 1,000 bp DNA ladder (Figure 2). To

Table 1. The Name and Sequence and Milting Point of Prepared Genes

Tm (ºC)	band sized	Sequence (5'–>3')	Gene Name
58	320 pb	F 5- CGG TGA CGT TCA GGT TCAC-3	*SOD*
		R 5- CAG CAC TAG CAG CAT GTT GAG C-3	

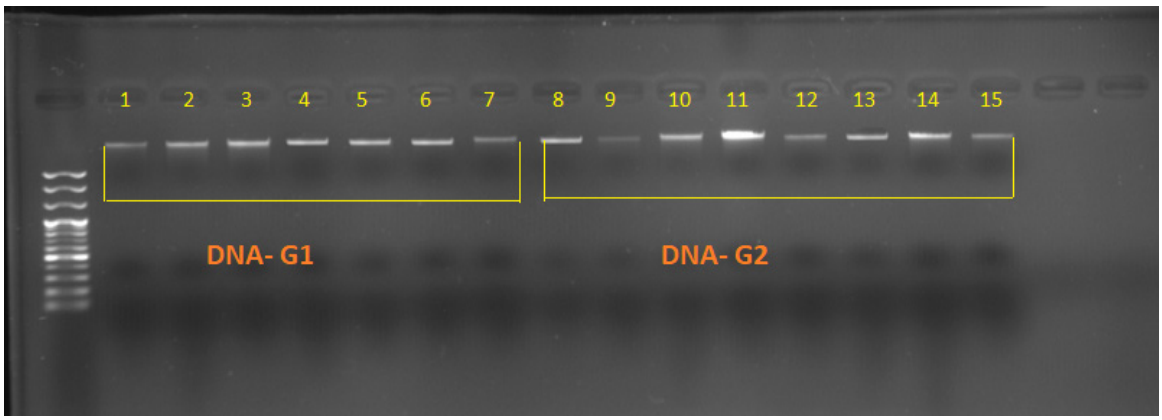

Figure 1. Gel Electrophoresis of Genomic DNA Extraction from Does. 1% agarose gel at 50 volt/cm for 45 min. M: DNA ladder (100-3000 bp) . Then visualized under U.V light after staining with green safe stain.

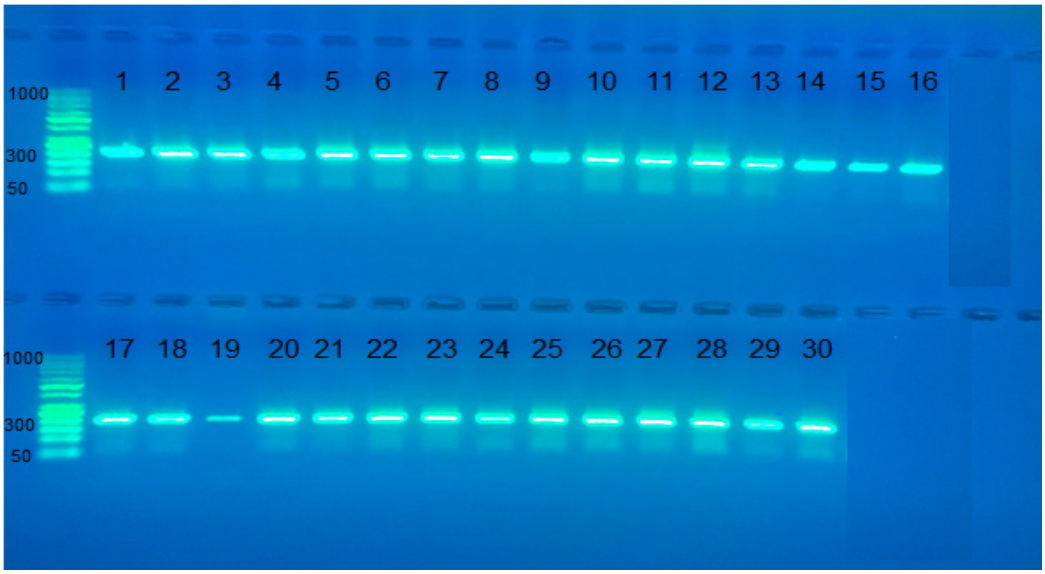

Figure 2. PCR Product of SOD2C47T Polymorphism the Band Sized 320bp. The product was electrophoresis on 1.5% agarose gel at 50 volts, 0.5x TBE buffer for 45 min. M: DNA ladder (50-1000bp), under U.V light after staining with safe stain.

Table 2. Basic Characteristics of Study Groups

Categories		Cases n=260 (mean ± SD)	Control n=295 (mean ± SD)	P value
Age		62.26±2.5	58.17±9.2	< 0.05
BMI		22.56±5.2	24.66±2.8	< 0.05
Sex	Male	172	204	< 0.05
	Female	88	91	< 0.05

do sequence analysis, the samples of five hundred and fifty fife PCR products were sent, 20 mL of PCR product was sent for each sample, and 100µL (10 pmol) was sent from the forward primer. The samples were prepared using an Applied Biosystems Program AB13730XL from the NICM / US business (Figure 3). The findings of the sequence analysis were analyzed using BLASTN server at the National Center for Biotechnology Information (NCBI). The results were linked to the NCBI's online available data from Gene Bank. To assess whether SOD2C47 T (T > C) was associated with susceptibility to lung cancer.

The polymorphic analysis revealed that all 3 potential genotypes (TT, TC, CC in SOD2C47T polymorphism) could be identified for these SNPs. The TT genotype was the main genotype in the participants tested at the locus of SOD2C47T polymorphism. The results showed no association between SOD2C47T polymorphism T and C-alleles and risk of lung cancer, where X2 is 3,574 and P=0,059 (Table 3).

In other words, this SNP and lung cancer had no significant relationship. The results also showed that the frequency of TT was 48.1% , TC was 33.2%, and CC was 18.7% in the control group. The frequency of TT was 43.5% , TC was 31.5% , and CC was 25% in the case group. There was no significant difference between groups in terms of SOD2C47T polymorphism (P = 0.168 >0.05) as shown in Table 3. In the control group, the frequency of T allele was 64.7% and that of C allele was 35.3%. In the case group, these frequencies were 59.2% and 40.8%,

Table 3. The Genotypes and Allele Distribution of SOD2 Polymorphism in G1(Control) and G2 (Patients)

Alleles SOD2C47T (T/C)	G1 (Control) N=295 (%)	G2 (Patients) N=260 (%)	OR (95%CI)	P value
TT	142 (48.1)	113 (43.5)	1.0^{ref} (1.0^{ref})	
TC	98 (33.2)	82 (31.5)	0.951 (0.648-1.396)	0.798
CC	55 (18.7)	65 (25.0)	0.673 (0.435-1.041)	0.075
T allele	382 (64.7)	308 (59.2)	1.0^{ref} (1.0^{ref})	
C allele	208 (35.3)	212 (40.8)	0.791 (0.620-1.009)	0.06
TT	142 (48.1)	113 (43.5)	1.0^{ref} (1.0^{ref})	
TC&CC	153 (51.9)	147 (56.5)	0.828 (0.592-1.158)	0.324
CC	55 (18.7)	65 (25.0)	1.0^{ref} (1.0^{ref})	
TC&TT	240 (88.6)	195 (75.0)	1.455 (0.969-2.182)	0.076

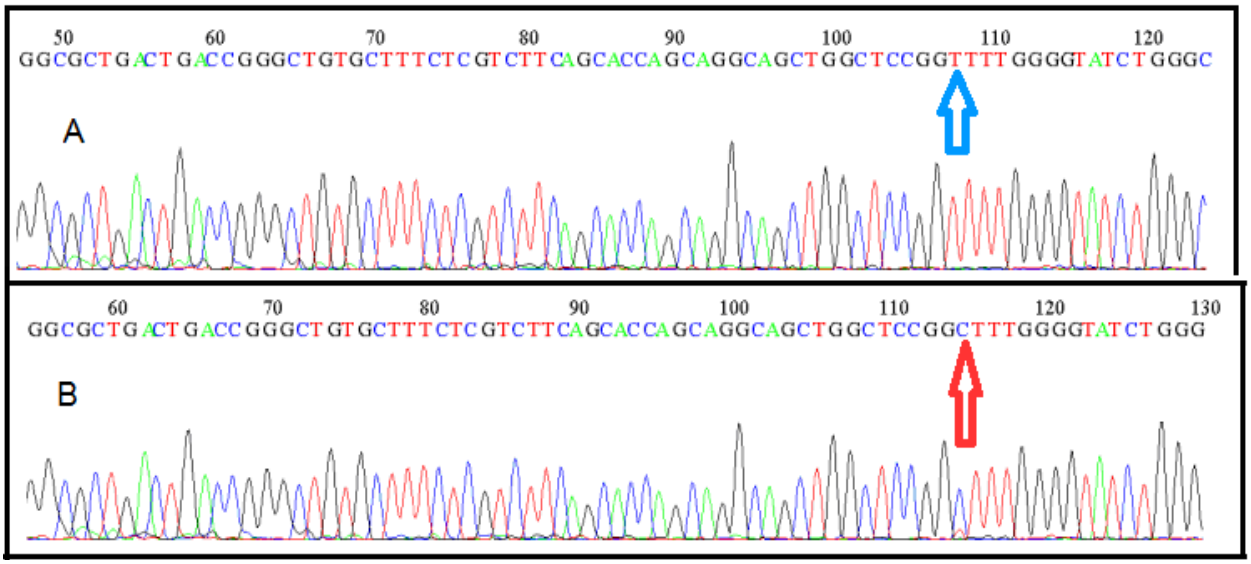

Figure 3. An Electropherogram of the Sequences Generated from PCR Products Amplified for SOD2C47T Polymorphism. There are two alleles showed in figures (T&C).

respectively. Descriptive statistical analyzes revealed that the polymorphism, TC, and CC genotypes of SOD2C47 T polymorphism were not significant compared to TT genotype (P = 0.270 > 0.05), and no significant difference was found between TC and TT genotypes compared to the CC genotype (P=0.370 > 0.05). As shown in Table 3, there was no significant relationship between C allele and the reference T allele, either (P=0.059).

Discussion

In this case-control analysis, we explored the possible association between polymorphisms in the *SOD2C47T* gene encoding polymorphism and lung cancer incidence. The findings showed that the polymorphism of SOD2C47T could not be a risk factor for lung cancer development.

Cell growth, cell division, and programmed cell death can be affected by free oxygen radicals. The activity of tumor suppressor genes can be decreased, and oncogenes may be triggered as a result of oxidative damage caused by reduced function or inadequate targeting of SOD2 (Carla et al 2009; Li et al., 2014).

Our study compared superoxide dismutase type 2 (SOD2) polymorphism in a healthy population and patients with lung cancer disorders. Our findings showed a lack of correlation between this polymorphism and any of the disease states. There are several studies investigated the relationship between SOD polymorphisms and incidence of lung cancer failing to validate any association between SOD polymorphisms and lung cancer, which is in contrast to this study. Lao et al., (2014) for example, revealed that this polymorphism decreased the risk of lung cancer.

Lung cancer is one of the most common cancers in Iraq. This research addressed the possible role of SOD2C47T polymorphism in developing lung cancer. Identifying the pathophysiology of lung cancer and understanding the causal factors are complicated and problematic.

The evidence indicates that both environmental and genetic factors contribute to lung cancer development. The main risk factors for this condition are smoking, family background, and genetic factors. Lung cancer is rare in individuals aged younger than 45 years old. People with a first-degree relative with lung cancer have a doubled risk of developing cancer compared to those with no family history (Xu et al., 2012; Zejnilovic et al., 2009)

Cigarette smoking is a well-ascertained risk factor for lung cancer (Wenzlaff et al., 2005; Xiaoying et al., 2019). As shown in, it has been found that cigarette smoking was associated with overall lung cancer risk. However, there is wide variability in individual responses to cigarette smoking. For example, heavy smoking is considered a high-risk factor for lung cancer, but only a small percentage of heavy smokers develop this disease. This suggests that some people may be hyper-susceptible and that this is potentially associated with genetic factors. Recent molecular biological studies demonstrated that the risk of bladder cancer due to cigarette smoking was precisely linked to genetic markers.

In line with previous studies, the results of this study indicated a lack of relationship between SOD2C47T polymorphism as a mutant homozygous genotype and the risk of lung cancer in Iraqi men.

This finding proves that cumulative of a genetic and occupational factor could highly increase bladder cancer risk more than only an occupational factor

Using descriptive analysis, no statistically significant relationship was detected between trend of lung cancer risk in (CT+TT) genotype of the SOD2C47T Polymorphism.

In conclusion, the current study suggested no association between SOD2C47T polymorphism and development of lung cancer. In addition, it was found that the combined influence of smoking and mutant gene enhanced the formation of lung cancer tumor. It was also found that the rate of developing and prognosis of the disease was profoundly affected by the mutation of the SOD2C47T polymorphism, which highlighted the role of

genetic marker in the diagnosis and monitoring of lung cancer tumors.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgments

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