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Association of TP53 codon 72 polymorphisms and non-small cell lung cancer risk in the Egyptian population : a case-control study

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Abstract: Lung cancer is considered the leading cause of cancer-related death worldwide. About 80% to 85% of lung cancers and the majority of cancer-related deaths worldwide were caused by the prominent subtype of non-small cell lung cancer. NSCLC displays several genetic abnormalities including the tumor suppressor gene (TP53). This study's objective was to determine if the TP53 codon 72 rs1042522 polymorphisms play a role in NSCLC susceptibility. Case-control research was done at the Mansoura university oncology center. The study included 124 patients suffer from NSCLC, and the control group included 124 healthy-matched volunteers. DNA from the blood was collected. To genotype single-nucleotide variations, ARMS-PCR was employed. The genetic study of TP53 rs1042522 (codon 72) showed Significant correlations among NSCLC cases and a larger percentage of AP, AP+PP genotypes, and P allele, ($p<0.05$ for each), with a risk to develop NSCLC (OR>1 for each). AP+PP genotype was significantly associated with lower vascular invasion when compared to the AA genotype. Otherwise, no significant association was found regarding TP53 genotypes with Tumor location, histopathology, or grade among all studied cases. In conclusion, our results showed that TP53 codon 72 rs1042522 polymorphism may be associated with the development of advanced NSCLC in Egyptians and can use as a prognostic biomarker for lung cancer.

Keywords: TP53; carcinoma; mutations

1. Introduction

With rising incidence and death, cancer continues to be a major public health issue. Lung cancer, which was virtually unknown first at end of the nineteenth century, has now become the most popular cancer worldwide. In 2020, there have been 10. Millions of cancer-related fatalities and 19.3 million new cases of cancer worldwide [1]**.** Histologically, Lung cancer is categorized into two types: (85%) of patients with NSCLC and the remaining (15%) is SCLC. The WHO has classed NSCLC into three major types: adenocarcinoma, large-cell lung cancer, and squamous cell carcinoma [2]. A few risk factors have a clear link to lung cancer besides cigarette smoking, which represents the second leading risk factor for early death and disability worldwide and remains the main determinant of the distribution of LC cases among genders and populations [3]. Those risks include residential radon from soil, accounting for 10 % of cases [4], and different origins of occupational exposures which account the third reason of lung cancer. Also, domestic fuel smoke [5] and chronic lung diseases, as chronic obstructive pulmonary disease (COPD), that have the

strongest link, particularly in men [6]. Asthma and sarcoidosis cause chronic inflammation inside the lungs. Moreover, observations have found a link between them and lung cancer risk [7], [8]. Importantly, the molecular genetic landscape of NSCLC is complex, with multiple underlying epigenetic and genetic mechanisms involved in carcinogenesis. NSCLC tumors have a wide range of chromosomal abnormalities, resulting in several protein fusions of the oncogenic type or copy number modifications including advancements and losses of crucial genes regulate cell cycle and chromatin remodeling [9], [10]. The p53 tumor-suppressor protein, encoded by the Tp53 gene is located at chromosome 17p13.1. p53 is either an inhibitor or a promoter of cancer cells. Approximately half of all human carcinomas harbor TP53 gene missense mutation giving rise to the full-length mutated p53 proteins with substitution of single-amino-acid [11]. Mutation in p53 (mutp53) has more complicated effects on expression of target genes and is thought to disrupt important signaling networks. Such altered p53 molecules not only lost their tumor-suppressive functions but also acquire additional oncogenic characteristics through a gain of function mechanism, which can give pre-cancerous colonies the ability for migration, invasion, as well as metastasis. [12].

Over 90% of TP53 variants are found in non-coding regions. Exons 5–8, encoding the DNA-binding domain, comprise the most commonly studied sites of mutation in TP53 [13]. Various diagnostic modalities are needed and common to reach a full assessment of advanced NSCLC. However, there is no marker or mixture of markers used for the early detection of LC in asymptomatic communities or elevated- risk groups such as smokers. The different tumor markers are useful in typing and monitoring the prognosis of the disease. There is currently no report in Egypt assessing the impact of Tp53 codon 72 mutation on advanced NSCLC by case-control study, which is preferable to cross-sectional studies in respect of risk factor evaluation. A comparative study was conducted in an Egyptian population of NSCLC patients and matched controls to assess the significance of this mutation in susceptibility to NSCLC in consideration of confounding factors such as the gender and the age differences between healthy and NSCLC patients and histopathological sub-types between NSCLC patients.

2. Materials and methods

The proposal was submitted to Mansoura Faculty of Medicine Institutional Research Board (MFM-IRB) for the approval (ethical code: MS.21.10.1721, date: 13/12/202). Patients and control participants signed informed consent forms. They were given a unique code in order to safeguard their privacy. The findings of this research might be published without disclosing any information about the subjects' identities.

Under this comparative study, 124 histologically proven NSCLC cases who were diagnosed or treated at the oncology Centre between 2021 and 2022 were paired with 124 normal participants of similar age and gender. Matching healthy controls were chosen based on their lack of clinical symptoms and a family background of NSCLC cancer and individuals who were not exposed to the original tumor or other intestinal illnesses. Nobody in the control group smokes, has a history of an illness that would interfere, or uses medicines on a regular basis. There were no unforeseen risks during the research. Waste materials were burned. In a hospital, the TNM, stage, tumor histology, and grade are examined. The molecular testing for genetic alterations was done in the lab.

Blood sampling

For the investigation of genetic mutation and hematological parameters, samples were collected by drawing 3 ml of the blood from all participants on (EDTA) tubes. After being collected, each sample was kept at -20°C. They were placed at room temperature before the technique to be used for DNA extraction. To find gene polymorphisms, then subjected to PCR analysis and gel electrophoresis.

Extraction of Genomic DNA

Leukocytes were isolated from samples of 2 mL from blood, and genetic Material was subsequently extracted using the common techniques of a commercial Easy Pure® DNA Purification kit (Transe GEN Easy Pure® Cat. No. EE121-01). Purity of DNA was measured via UV light absorption spectrometric at

260 nm wavelength. Each specimen was set at 25 ng/L in preparation for genotyping.

TP53 codon Arg 72 Pro (rs1042522) ARMS-PCR:

In a single PCR reaction, four primers have been utilized. To amplify the 281 bp band, that serves as a control band, two primers, P1 and P2, were used. As shown in table (1), two unique primers having complementary 3' termination nucleotides to the matching polymorphism were developed. For each , a destabilizing mismatch was added at the third nucleotide from of the 3'-terminus to increase specificity [15]

Table (1): Primer pairs used for screening of TP53 codon Arg 72 Pro (rs1042522) mutation by ARMS-PCR

Mutation	Primer sequence	Size (bp)
TP53 Arg 72 Pro (rs1042522)	P1: 5´-GCCGTCCCAAGCAATGGATGATT-3´	281bp
	P2: 5´-GGCAACTGACCGTGCAAGTCACAG-3´	
	P3: 5´-AGAATGCCAGAGGCTGCTCCACC-3´	193bp
	P4: 5´-CCTCTGGTGCAGGGGCCAAGC-3´	

A total amount of 30 µl, including 8 µl of external primers, was used for each PCR reaction mixture (4 µl of p1 and 4µl of p2), 16 µl of master mix (COSMO PCR RED Master Mix **(W10203001)),** Mixed with 4 µl of DNA in a thin-walled PCR tube. 4 µl of p3 and µl p4 were added. Samples were amplified by the usage of T professional thermocycler (Biometra, Germany). The PCR assay conditions were: initial denaturation 94°C, 5 minutes for 1 cycle; 36 cycle including denaturation at 94°C, 30 second; annealing 65°C, 30 second; 72°C for extension, for 50 seconds; and final extension at 72°C, for 5 minutes, in 1cycle; then soak at 4°C.

The products of PCR were electrophoresed on 2.5 % agarose gel. They were visualized under UV. The procedure rendered three bands in the heterozygote (281,193 and131 bp), two bands in the homozygote (Arg/Arg result in 281 and 131 bp) or (Pro/Pro result in 281 and 193 bp).

Statistics:

The data collected were analyzed and tabulated using the SPSS software package (IBM Corp. 2017. windows SPSS Statistics, Version 25.0. Armonk, NY: IBM Corp.). As regard to demographic and clinical characteristics of the study population, categorical variables including gender are presented as frequencies with percentage. 95% confidence interval and odd ratio (OR) conferred by potential correlations, regarding the TP53 gene polymorphisms with the risk and progression of NSCLC. The probability level (P) of less than 0.05 was defined as a criterion of significance. Hardy-Weinberg equilibrium (HWE) for the SNP was calculated by goodness-of-fit between the observed and expected genotype frequencies

3. Result and discussion:

All examined genotypes in the healthy controls and in NSCLC patients were in HW equilibrium, according to the Weinberg specific equation, no significant differences were found between observed and expected counts in each group. The results presented here compare selected data of all investigated parameters between cases of cancer patients and control persons. Patients were 50 women and 74 men, the men constituted the majority of cases. The average (±SD) ages of the patients were 56.0 (±11.5) years. 124 healthy adults, including 78 men and 46 women, made up the control group. The average age (±SD) of the controls were 55.4 (±11.0) years. Both patients and healthy controls appeared to be homogenous regarding age and (P=0.272 and 0.602, respectively). Table (2)

Table (2). Comparison of age and gender among studied groups

	Control n=124		**Casesn=124**		**P**
Age mean± SD	554	±11.0	56	±11.5	0.272
Males n(%)	78	64.0%	74	59.7%	0.602
Females n(%)	46	36.0%	50	40.3%	

SD, standard deviation; The t-test and X chi-square are employed to compare numerical information reported as mean and SD.

Table (3) shows a very statistically significant difference ($p<0.05$) in TP53 genotype polymorphisms from controls. concerning the rs1042522 polymorphic genotype, the AP heterozygous genotype was found in 52 NSCLC patients (41.9%) higher than in controls which appear in 26 (21.0%). The PP homozygous genotype was observed in only 3 (2.4%) of NSCLC patients and no one in the healthy controls. while the AA homozygous genotype was lower in patients 69 (55.6%) than in the control 98 (79.0%). AP genotype increased probability of NSCLC (Odds ratio [OR], 1.933; ($P<0.001$)). Moreover, (AP+PP) genotyping model, have a statistical association with NSCLC ([OR], 2.437; ($P=0.003$)) in comparing to the AA genotype (wild type). In compared to the A allele (wild type), the P allele which represents 58 (23.4%) of NSCLC and 26 (10.5%) of healthy volunteers, was linked with a higher incidence of NSCLC ([OR], 1.860; ($P<0.001$)). **Figure (1).**

Table (3): Comparison of TP53 rs1042522 genotypes frequency and alleles between NSCLC cases and healthy volunteers.

Genetic model	genotype	Control (124 n)		NSCLC (124 n)		p	OR	(95% CI)
		n	%	n	%			
Reference	**AA**	98	79.0	69	55.6	Wild type		
heterozygous	**AP**	26	21.0	52	41.9	**<0.001**	1.933	(1.368-2.733)
homozygous	**PP**	0	.0	3	2.4	1		
Dominant	**AP+PP**	26	21	55	44.3	**0.003**	2.437	(1.351-4.395)
Allelic	**A**	222	89.5	190	76.6	Reference		
	P	26	10.5	58	23.4	**<0.001**	1.860	(1.341-2.579)

P, probability; $p<0.05$ is significant Odds ratio [OR]; Confidence interval [CI]

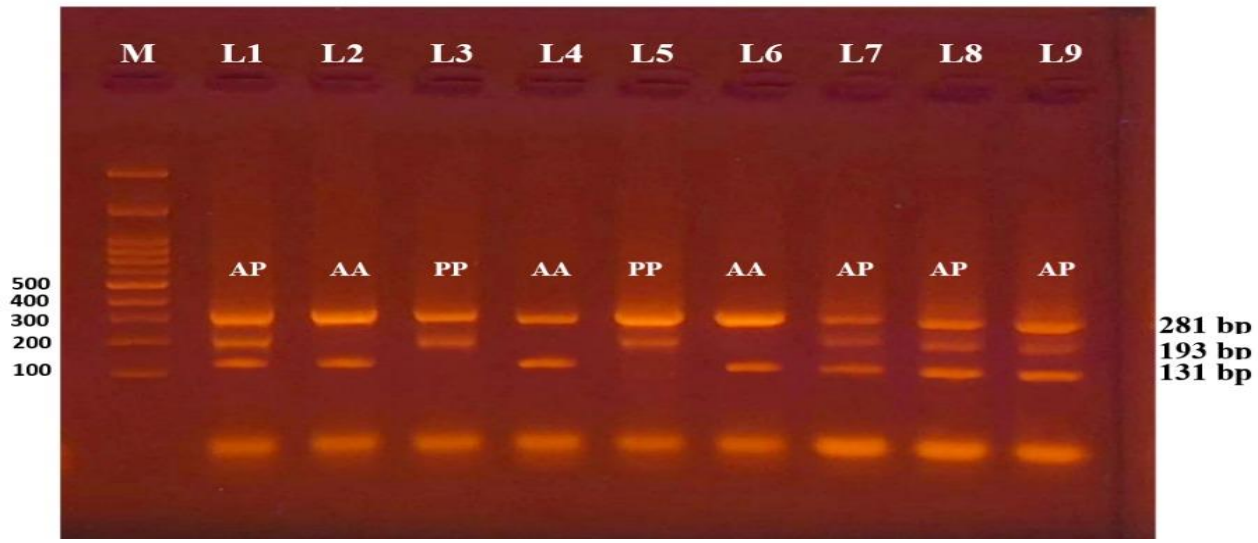

Figure (1): Gel electrophoresis of TP53 gene Arg 72 Pro (rs1042522) BY tetra-ARMS PCR, where each lane represents one participant. M stands for DNA ladder (100 bp). The internal control is shown by the 281 bp band, specific 193 bp bands represent the P(Proline) allele, and specific 131 bp bands represent A (Adenine) allele. AP heterozygous is represented by lanes 1 and 7, 8, and lane 9. Lanes 2,4, and 6 indicate AA homozygous; whereas the P allele is absent, and the A allele is present at 131bp. Lanes 3 and 5 represent PP homozygous, with the P allele appears at 193 bp and the A allele absents

The histology and grading of the malignant cells should be used to select instances for investigation. Of the 124 individuals studied, 98 (79.0%) had adenocarcinomas, 12 (9.7%) had large cell carcinomas, 8 (6.5%) had squamous cell carcinomas, while 6 (4.8%) had other forms of NSCLC. In terms of the staging, 77(64.2%) of cases were classified as the 3rd grade, 41(34.2%) were within the 2nd grade, and just 2(1.7%) were in the primary grade. The overall frequency of TP53 (Arg72Pro) mutation (AP+PP) found in in adenocarcinoma was 72.7%% (40 of 55), large cell carcinoma was 12.7% (7 of 55), squamous cell carcinoma was 9.1% (5 of 55),

and other NSCLC subtypes was 5.5% (3 of 55). No significant correlation was seen

between the presence of the point mutation and the histological subtype (p=0.454) as well as grade (p=0.414), according to two-sided testing. **Table (4).** Tumor characteristics including its location, multiplicity, density and vascular invasion was investigated. Regarding TP53 rs1042522, AA genotype (wild type) was significantly associated with vascular invasion (p=0.013) comparing to AP and PP genotype. Otherwise, no significant association was found regarding TP53 genotypes with Tumor characteristics among all studied cases while there was no association between other parameters and TP53 mutation among all studied cases. **Table (5).**

Table (4): Association of TP53 genotypes with Tumor pathology among all studied NSCLC cases.

TP 53 (Arg 72 Pro) histopathology		**AA**		**AP+PP**		***P***
		n	%	n	%	0.454
Pathology	adenocarcinoma	58	84.1%	40	72.7%	
	large cell carcinoma	5	7.2%	7	12.7%	
	squamous cell	3	4.3%	5	9.1%	
	Others	3	4.3%	3	5.5%	
Grade	1	2	3.0%	0	0.0%	0.414
	2	25	37.3%	16	30.2%	
	3	40	59.7%	37	69.8%	

Table (5): Association of TP53 genotypes with Tumor characteristics among all studied

TP 53 Arg 72 Pro		AA		AP		PP		AP+PP		P1	P2
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**		
Tumor location	**Left**	17	24.6%	19	36.5%	0	0%	19	34.5%	0.344	0.457
	Right	28	40.6%	20	38.5%	1	33.3%	21	38.2%		
	Bilateral	24	34.8%	13	25.0%	2	66.7%	15	27.3%		
Number	**Single**	27	39.1%	22	42.3%	1	33.3%	23	41.8%	0.935	0.762
	Multiple	42	60.9%	30	57.7%	2	66.7%	32	58.2%		
Density	**Cystic**	2	3.0%	5	10.6%	1	33.3%	6	12.0%	0.151	0.072
	Solid	65	97.0%	42	89.4%	2	66.7%	44	88.0%		
Vascular invasion		20	29.0%	4	7.8%	0	0%	4	7.4%	0.013	0.003

P1, comparison between AA, AP and PP; *P2*, comparison between AP+PP versus AA.

Cancers in humans are brought on by various genetic changes in the TP53 (#191117 OMIM). For instance, somatic mutations are frequent throughout most cancers. The antiproliferative activity of the p53 protein in response to diverse stresses, as well as throughout physiological mechanisms such as senesce, renders it a top target for cancer suppression [16]. Various researches investigating the role of TP53 genetic variants in LC have yielded conflicting results, despite the fact that many studies had also observed that people who are polymorphic for tumor suppression or repair genes have an increased likelihood of developing LC. The most frequently investigated variant (SNP) being a G/C difference inside exon 4 within codon 72, resulting in Pro72 or Arg72 protein [17]**.** Codon 72 is required for apoptotic cell death and anticarcinogenic. It is found within the proline-rich area of the p53 protein. Because the Arg72 variation of the p53 protein performs better inside the mitochondria than the Pro72 variant, Arg72 has a fifteen-fold greater capacity to trigger apoptosis. Thus, the Pro/Pro variant is believed to be accountable for reduced apoptosis which contributes to cancer development [18]**.** For this study, the distribution of the heterozygous genotype Arg/Pro and dominant AP+PP of TP53 rs1042522, show a very statistically significant difference from control participants ($P< 0.001$ for AP, and $P = 0.003$ for dominant) compared to AA reference dominant model. Moreover, allelic P model shows a significant correlation with NSCLC with ($P < 0.001$) and odd ratio >1. This investigation is congruent with the findings reported by Chowdhury [19] who observed a significant linkage of TP53 rs1042522 genetic polymorphism with LC higher susceptibility in the Bangladeshi community. A previous meta-analysis for African Americans concluded that common genetic variation in TP53 could associate with

LC [20]**.** Although, new case-control study indicate that TP53 rs1042522 variant was not related to risk of LC in Patients from Bangladesh [21]**.** Statistical analyses of a previous study in a Korean population demonstrated that genetic variants and haplotypes inside the TP53 gene, which include Arg72Pro, were not really significantly related to LC [22]**.** The discrepancy in results between the current study and the two previous studies could be attributed to sample size, variability in histological subtypes and diagnostic stages of LC, as well as the age of the subjects studied. According to a previous study, NSCLC, especially SQC and AC, are the commonest types of LC in Bangladesh were associated with codon 72 polymorphisms [23]**.** However, our findings show no link between codon 72 mutations and the hazard of any histopathology subtype (P= 0.454) or grade (P= 0.0.414) of NSCLC. An Asian study within the same site demonstrated no association with SQC or AC (P>0.05) with codon 72 genotypic models but also an important significant result with P allele regarding SQC and AC (P<0.01) [19]. Different sex and ethnic background might explain Conflicting results. In comparison with the AP and PP genotypes, the AA genotype (wild type) was significantly linked with Vascular invasion (P=0.013). Nevertheless, A recent study conducted in Bangladesh found no link between these genetic variations and the occurrence of more aggressive cancers in the late stages [21]**.**

In conclusion, A deeper awareness of the tumor's biology and the most predominant genetic mutation in TP53 gene associated within Egyptian society allows for disease prediction before critical stages and the provision of targeted treatments that promise to increase the chances of survival from lung cancer. Our findings suggest that the TP53 SNP rs1042522 genotypes can be used to predict NSCLC disease.

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