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Original Research

Association of *P53* codon 72 polymorphism and lung cancer in an ethnic Iranian population

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Abstract: Lung cancer is one of the most common causes of cancer death worldwide. Molecular genetic studies indicated that activation of dominant oncogenes or inactivation of tumor suppressor genes and the presence of polymorphism in these genes correlated with prevalence of new lung cancers. *P53* as a tumor suppressor gene located at 17p13 chromosome and it is one of the most well-known mutant genes in all cancer types. Mutation in *P53* can disturb the transcriptional function and suppression of cell cycle control and increase in cell division and amplification. We can predict the susceptibility of people inside a society to lung cancer with evaluation of *P53* gene polymorphism. A total of 200 patients with lung cancer and 200 healthy controls participated in this case-control study. Genomic DNA was extracted from blood samples and PCR-RFLP analyses were used to genotype the *P53* gene polymorphism in codon 72 of exon 4, chromosome 17. Among 200 lung cancer patients and 200 controls, there was no significant correlation between sexuality and cigarette smoking status. We did not find any relationship between cigarette smoking status and genotypes or pack-years but there was a significant correlation between cigarette smoking status and adenocarcinoma patients (P=0.03). The results of the present study revealed that there is no association between *P53* codon 72 polymorphism and increased risk of lung cancer in patients and controls but according to results of adenocarcinoma in never-smoker patients, it seems that environmental factors may have more important role than genetic susceptibility in our ethnic Iranian population.

Key words: Lung cancer, adenocarcinoma, squamous cell carcinoma, small cell carcinoma, polymorphism, *P53* gene.

Introduction

Lung cancer is a serious problem and one of the most common malignant diseases and leading cause of cancer death worldwide and during these years the mortality rate of lung cancer have risen into epidemic proportions in Western world (1, 2). It seems that tobacco smoking accounts for the majority of lung cancer but some patients with lung cancer are lifelong never smokers (3). Two main types of lung cancer are small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC). According to histopathological classification of lung cancer, NSCLC divided into three main subtypes: adenocarcinoma, squamous-cell carcinoma and large-cell carcinoma. The histologic distinction between NSCLC and SCLC is clinically extremely important. There are considerable differences between those two groups in both, therapeutic approach and prognosis of the disease (4). Genetic polymorphisms of the tumor suppressor genes are implicated into the susceptibility of cancer (5). Among the genetic factors, the *TP53* tumor suppressor gene is mutated in many forms of human cancers (6). A common polymorphism occurs at codon 72 that is located on the exon 4 of chromosome 17 and substitution of the G in arginine (CGC) to C in proline (CCC) is a risk factor for the incidence of such cancers like breast, endometrium and lung (7, 8). An association between *P53* codon 72 polymorphism and lung cancer was first reported by Weston and co-workers in 1992 among U.S. populations (9) and after that many studies evaluated the association of *P53* codon 72 polymorphism and lung cancer risk; no clear consensus, however, was reached (10). In an Indian study, the *Arg/Pro* genotype in patients with lung cancer was associated with early progression of the disease, compared with *Arg/Arg* carriers (11). Another meta-analysis conducted by Matakidou *et al*. (12) described that there was no relationship between TP53 codon 72 polymorphisms and risk of lung cancer. Dumont *et al.* (13) suggested that the TP53 proline 72 variant is associated with increased risk of cancer due to a decreased ability to induce apoptosis. In the present study, we conducted a case-control study of lung cancer patients and evaluated the genotype frequency of codon 72 of lung cancer patients and controls, using PCR-RFLP based genotyping methods to further evaluate the possible relevance of this polymorphism for lung cancer risk.

Materials and Methods

Subjects

This study was a case-control hospital-based study that included 200 lung cancer patients (mean age 53 ± 11) and 200 healthy controls (mean age 50 ± 10). All lung cancer patients were admitted in Ghaem hospital, Mashhad University of Medical Sciences, Mashhad, Iran between February 2009 and December 2015. The controls were from healthy blood donors with no signs

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and symptoms of hematologic disorders, autoimmune disease or malignancy. The study was approved by the Ethical Committee of Mashhad University of Medical Science. All subjects gave their informed consent.

Genotyping

Whole blood was collected from the enrolling subjects and genotyping of lung cancer patients and healthy controls was performed by Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP). DNA was isolated from peripheral blood cells using a standard salting out protocol (14,15). A fragment of DNA around exon 4 of the *P53* gene (the location of the codon 72) was PCR-amplified using the following primer pair: 5'-TTGC-CGTCCCAAGCAATGGATGA-3´ as forward and 5´-TCTGGGAAGGGACAGAAGATGAC-3´ as reverse primers as described previously (16) were yielding a single 199 base pair (bp) product. The reaction mixture contained approximately 100 ng genomic DNA, 2 mM of each primer, 0.2 mm of each dNTP, 1.5mm $MgCl_2$, and 1U of *Taq* polymerase (MBI Fermentas). PCR amplification consisted of an initial 5 min denaturation at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The terminal extension was performed at 72 °C for 10 min. Then, the PCR product (199 bp) (Fig. 1) was digested with 2 units of restriction enzyme *BstUI* at 60 °C for 16 h. DNA fragments were identified in a 1.4% agarose (MBI Fermentas) gel stained with ethidium bromide.

Statistical analysis

Univariate statistics (χ^2 and t tests) were used first to compare cases and controls for demographic variables and genotype prevalence. Statistical analysis of the data was performed by SPSS version 20.0 (Spss Inc, Chicago, Il, USA) software.

Results

The distribution of demographic variables for cases and controls is summarized in Table 1. The mean ages were 53 years for cases and 50 years for controls. In this study, control group was more likely to be current smokers (46% for controls versus 29% for cases) and to have accumulated more pack-years than the lung cancer patients (47.7 for control group versus 46.5 for case group). According to Table 2, the results showed that *Arg/Arg* genotype appeared in 48.5% and 44% of the patients and controls, respectively; the *Pro/Pro* genotype in 10% and 12%; and the *Arg/Pro* genotype in 41.5% and 44%. So, there was no significant differences between genotypes of patient and control groups (P=0.7). The frequencies of the three genotypes, *Arg/Arg*, *Arg/Pro*, and *Pro/Pro* were evaluated by lung cancer histopathological subtypes. The distribution of the three genotypes in squamous cell, small cell, large cell and bronchi alveolar cell carcinoma patients showed a significant increase in *Arg/Arg* genotype and a decrease in *Pro/Pro* genotype (P=0.03). But in adenocarcinoma, *Arg/Pro* (54.4%) genotype expressed more than *Arg/Arg* (30.3%) genotype while *Pro/Pro* had the least frequency. Patients grouped by smoking habit showed that there was no significant differences between cigarette smoking statuses (Current smoker, P=0.7; Ex-smoker, P=0.4; Never smoker, P=0.2) in cases and controls.

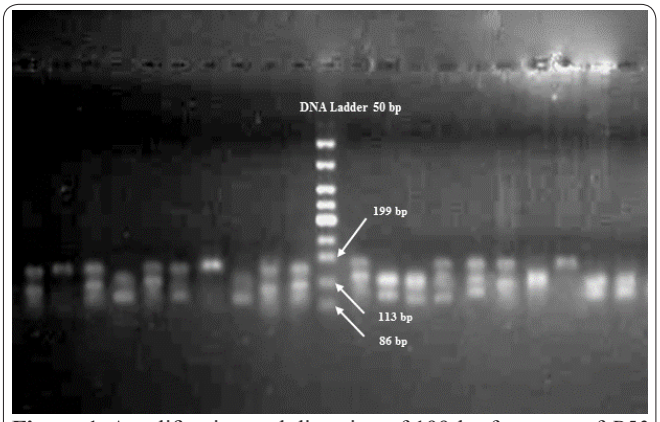

Figure 1. Amplification and digestion of 199-bp fragment of *P53* gene by PCR-RFLP method. The *Arg* allele is cleaved by *Bst*UI and yields two small fragments (113 and 86 bp). The *Pro* allele is not digested by *Bst*UI at codon 72 and has a single 199-bp band. The *Arg/Pro* genotype has three bands (199, 113, and 86 bp). The 50-bp DNA ladder in the central lane is labeled.

Table 1. General characteristics of selected variables between cases and controls in the whole study population.

Characteristic		Case (n=200)	Control (n=200)
Sex			
	Male	112 (56%)	92 (46%)
	Female	88 (44%)	108 (54%)
Age, mean (years)		53 ± 11	50 ± 10
Smoking status			
	Never smoker[a]	98 (49%)	84 (42%)
	Ex-smoker[b]	44 (22%)	24 (12%)
	Current smoker[c]	58 (29%)	92 (46%)
Pack-years, mean		46.57	47.7
Cigarette per day, mean		31.65	19.95
Education			
	High-school graduate	128 (64%)	172 (86%)
	<High-school graduate	72 (36%)	28 (14%)

[a] Less than 0.05 pack-years in lifetime.

[b] Quit smoking at least 1 year before enrollment.

[c] Smoked at least one cigarette per day for at least 1 year or 20 packs of cigarettes.

Table 2. Genotype frequencies of *P53* codon 72 polymorphism among controls and histopathological types of lung cancer patients.

	n	*Arg/Arg* n (%)	*Arg/Pro* n (%)	*Pro/Pro* n (%)	P-Value
Control	200	88 (44.0)	88 (44.0)	24 (12.0)	
Histological types					
Adenocarcinoma	79	24 (30.3)	43 (54.4)	12 (15.0)	0.7[a]
Squamous cell	37	21 (56.7)	12 (32.4)	4 (11.1)	
Small cell	68	40 (58.8)	24 (35.3)	4 (5.90)	
Large cell	5	5 (100)	0 (0.00)	0 (0.00)	
Broncho-alveolar	11	7 (63.6)	4 (36.4)	0 (0.00)	
All	200	97 (48.5)	83 (41.5)	20 (10.0)	

[a] P=0.7 for comparison of adenocarcinoma with other histopathological types of lung cancer.

Frequency distribution of cigarette smoking status and histopathological types of lung cancer showed that only the number of never smoker patients with adenocarcinoma were significantly higher than the other groups (χ^2=19.9, P=0.03) (Table 3). Our results showed that *Arg/Pro* genotype in never smoker adenocarcinoma patients was significantly higher than *Arg/Arg* and *Pro/Pro* genotypes (P=0.04) but in our control groups there were no significant differences between cigarette smoking status and genotypes mentioned above (P=0.3) (Table 4). Our results showed no correlation between histopathological types of lung cancer and smoking exposure based on pack-years (P=0.9).

Discussion

Today, lung cancer and respiratory infectious diseases are significant public health concerns in Iran. Several factors, such as cigarette smoking, fossil fuel exhausts, particulate pollutants and tuberculosis increase the risk of lung cancer. There are many reports which aimed to evaluate the relationship between *P53* gene codon 72 polymorphism and risk of different cancers such as colon, lung, esophagus, breast, liver and prostate (17-19). Several studies have shown that *P53* polymorphism is segregated differentially among different ethnic populations, the *Arg* allele being more common in Caucasian than in African or Asiatic populations (20). The relationship between genes and lung cancer might be susceptible in different ethnicity and also the interaction of genes and environmental factors could have an important role in susceptibility to lung cancer. So, according to geographical distribution of *P53* codon 72 it seems to act as a genetic susceptibility marker (10). We analyzed the distribution of the *P53* codon 72 polymorphism genotypes *Arg/Arg*, *Arg/Pro* and *Pro/Pro* in

Table 3. Frequency distribution of cigarette smoking status and histological types of lung cancer.

	Never smoker n (%)	Ex-smoker n (%)	Current smoker n (%)	P-Value
Control	84 (42.0)	24 (12.0)	92 (46.0)	
Histological types				
Adenocarcinoma	54 (27.0)	10 (5.0)	15 (7.5)	0.03[a]
Squamous cell	12 (6.0)	17 (8.5)	8 (4.0)	
Small cell	21 (10.5)	12 (6.0)	35 (17.5)	
Large cell	5 (2.5)	0 (0.00)	0 (0.00)	
Broncho-alveolar	6 (3.0)	5 (2.5)	0 (0.00)	
All	98 (49.0)	44 (22.0)	58 (29.0)	

[a] P=0.03 for comparison of adenocarcinoma with other histopathological types of lung cancer.

Table 4. Frequency distribution of *P53* genotypes and cigarette smoking status in Adenocarcinoma patients and control groups.

	Arg/Arg n (%)	*Arg/Pro* n (%)	*Pro/Pro* n (%)	P-Value
Control				
Never-smoker	36 (18.0)	37 (18.5)	11 (5.5)	0.3[a]
Ex-smoker	11 (5.5)	8 (4.0)	5 (2.5)	
Current smoker	41 (20.5)	43 (21.5)	8 (4.0)	
Adenocarcinoma				
Never-smoker	12 (15.2)	38 (48.1)	4 (5.1)	0.04[b]
Ex-smoker	8 (10.1)	2 (2.5)	0 (0.0)	
Current smoker	4 (5.1)	3 (3.8)	8 (10.1)	

[a] P=0.3 for comparison of *P53* genotypes and cigarette smoking status in control group.

[b] P=0.04 for comparison of *P53 Arg/Pro* genotype with other genotypes in different cigarette smoking status of adenocarcinoma patients.

our case and control samples. According to frequencies of *Arg/Arg*, *Arg/Pro* and *Pro/Pro* genotypes in our study, we found that there is no significant difference between lung cancer patients and controls. Chowdhury *et al*. (2015) found no significant differences in relative risk of developing lung cancer for having *Pro/Pro* genotype (P=0.33) or *Arg/Arg* genotype (P=0.2) (16), however Fan and colleagues (2000) observed that there were significant differences between these genotypes in case and control groups (21). The results of this study showed that *Arg/Arg* genotype is expressed more than other genotypes in squamous cell, small cell, large cell and bronchi alveolar cell carcinoma patients, however *Arg/Pro* was the dominant genotype in adenocarcinoma. In all lung cancer patients *Pro/Pro* genotype had the least frequency. The results of one meta-analysis in China showed that the *Pro/Pro* and *Pro* carrier may be protective factors for lung cancer in the southern China whereas risk factors in the northern China, suggesting a possible role of geographical differences in the environment they lived in (10). However, the results of one study in Bangladesh demonstrated there was no association of codon 72 polymorphisms and the risk for small cell lung cancer (P=0.66). However, the results also indicate that the *Pro* allele is a significant risk factor for adenocarcinoma (P<0.01) and squamous cell carcinoma (P<0.01) (16). Many studies reported that polymorphism of *Arg*/*Pro* has been linked to cancer susceptibility, notably lung, breast and colon cancer, however, these findings remain controversial (22, 23). For instance, the study of Jones *et al*. indicated that *Arg*/*Pro* genotype developed colorectal cancer 13 years earlier than *Arg*/*Arg* (24). In our study, we found that never-smoker adenocarcinoma patients were significantly more that ex-smoker or current smoker lung cancer patients and according to other reports these findings were controversial. For instance, Devi *et al.* (2010) observed the association of the *Arg/Pro* genotype with an increased risk of lung cancer among current and ex-smokers not for never smokers (25). It seemed that the high rate of lung cancer in our never smoker patients related to other factors like air pollution or maybe they were passive smokers. We did not find any relationship between pack-year cigarette smoking and genotypes in adenocarcinoma patients but some reports indicated that the frequency of the *Pro/Pro* genotype in adenocarcinoma was much lower than that of the *Arg/Arg* genotype in the low pack-years stratum and the prevalence of the *Pro/Pro* genotype rose linearly with pack-years (21). On the other hand, Chowdhury *et al*. (2015) showed no relationship between increasing pack-year and lung cancer risk (16).

Our study showed that the *P53* codon 72 polymorphism is not associated with an overall increased risk of lung cancer in Iranian patients. We did not find any relationship between cigarette smoking status and genotypes or pack-years but according to the results of our study, it seems that other factors like environmental exposure to smoke or air pollution might have more important role than genetic susceptibility in our adenocarcinoma lung cancer patients. Further studies, with larger numbers of patients, are required to determine the contribution of other clinic pathological parameters of the diseases with the *P53* codon 72 polymorphism.

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