Lung Cancer (2007) **56**, 145—151

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/lungcan

An increased lung cancer risk associated with codon 72 polymorphism in the TP53 gene and human papillomavirus infection in Mazandaran province, Iran

Seyed Alireza Nadji[a,b,*], Mahmood Mahmoodi[a], Abed Ali Ziaee[c], Farshad Naghshvar[d], Jila Torabizadeh[d], Yousef Yahyapour[a], Rakhshandeh Nategh[a], Talat Mokhtari-Azad[a]

[a] *Virology Division, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[b] *Masih Daneshvari Hospital, NRITLD, Shaheed Beheshti University of Medical Sciences, Tehran, Iran*
[c] *Institute of Biochemistry and Biophysics, Tehran University, Tehran, Iran*
[d] *Imam Khomeini Hospital, Department of Pathology, Mazandaran University of Medical Sciences, Sari, Iran*

Received 17 June 2006; received in revised form 1 December 2006; accepted 6 December 2006

KEYWORDS
TP53 gene;
Codon 72 polymorphism;
Lung cancer risk;
Human papillomavirus;
Iran

Summary The TP53 gene has a polymorphism in exon 4 at codon 72 that presents the arginine or proline genotype. The association of TP53 codon 72 polymorphism with lung cancer risk has been studied by several groups, although with inconsistent results. Our previous study showed that the human papillomavirus (HPV) infection is associated with the development of lung cancer in Mazandaran, north part of Iran (cases = 25.6% versus controls = 9.0%, $P = 0.002$). The frequency of TP53 codon 72 polymorphism was studied in a north part Iranian group of 92 healthy controls and 141 lung cancer patients. The allelic distribution of the three genotypes (ArgArg, ArgPro, ProPro) in healthy normal controls was 46.1, 32.6 and 21.3%, respectively, which differs from that of lung cancer patients showing genotype frequency as 42.6, 49.6 and 7.8%. A relation between the presence of the Arg allele and lung cancer risk was observed. Our study reveals that Arg allele, active smoking and HPV infection are the important risk factors in lung cancer development in the north part of Iran, Mazandaran province.

Abbreviations: Arg, arginine; Pro, proline; RR, arginine/arginine; RP, arginine/proline; PP, proline/proline; HPV, human papillomavirus; PCR, polymerase chain reaction; S.D., standard deviation; OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval; NSCLC, non-small cell lung carcinoma; AdC, adenocarcinoma; SCC, squamouse cell carcinoma; LCC, large cell carcinoma; SCLC, small cell lung carcinoma

* Corresponding author at: Virology Division, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. Tel.: +98 9123802639; fax: +98 21 88736077.
E-mail address: sarnadji@razi.tums.ac.ir (S.A. Nadji).

0169-5002/$ — see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2006.12.006

1. Introduction

Lung cancer is the leading cause of cancer deaths in the world [1] and the vast majority of cases are smoking related. Genetic polymorphisms in the genes involved in tumourigenesis may determine individual susceptibility to cancer. To date, several polymorphisms have been described in the wild-type TP53 gene [2—5].

Germline TP53 mutations have been reported to be associated with inherited breast cancer risk [6], and codon 72 polymorphism on the exon 4 of the TP53 gene, which encodes variant proteins with an arginine (CGC) or proline (CCC), have also been studied as potential susceptible genotypes for lung cancer [7—10].

The genotype distribution of TP53 codon 72 polymorphism is significantly different among ethnic groups. Beckman et al. [11] reported that there was a significant decrease in the frequency of the *Pro* allele with increasing latitude, ranging from 0.63 in African Blacks to 0.17 in Swedish Saamis. Weston et al. [12] also reported that the frequency of the *Pro* allele varied by ethnicity. The TP53 *Pro* allele was found to be more common in African-Americans (0.50) than in Caucasians (0.29). Two Japanese studies showed genotype frequencies of *Pro* ranging from 0.35 to 0.40 [8,9]. The association of TP53 codon 72 polymorphism with lung cancer risk has been studied by several groups, although with inconsistent results [7—10,12,13].

In our previous study, we analysed the association between HPV infection and lung cancer development [14]. These results suggested that HPV infection is associated with the development of lung cancer in Mazandaran, the north part of Iran, and the low prevalence of HPV infection in lung cancer is leaving room for agents other than HPV infection.

The genotype distribution of TP53 codon 72 polymorphism remains undefined in the Iranian population. Hence in the present study, we conducted a case-control study of lung cancer patients and controls and examined the genotype frequency of codon 72 polymorphism in them using PCR-based genotyping methods to evaluate the possible relevance of this polymorphism for lung cancer risk among north Iranian population from Mazandaran province.

2. Materials and methods

2.1. Tissue samples

A total of 233 blocks of paraffin-embedded tissue including 141 samples diagnosed as lung carcinomas and 92 non-cancer samples as control were retrieved from archive of Imam Khomeini Hospital, Medicine faculty of Sari city, Mazandaran University of Medical Sciences, Iran between 1998 and 2004. The low number of controls, comparing the number of cases is due to limitation we had in accessing the controls with suitable characteristics. The non-cancer patients with different lung diseases, including pneumothorax, crypto-coccal and hydatic cyst infection and fibrosis served as control subjects. Furthermore to adjust the environmental confounders, we tried to match the subject's residence place in Mazandaran province between both groups.

2.2. DNA extraction and PCR

Genomic DNAs from tissue sections were prepared according to the methods described by Impraim et al. [15]. To avoid contamination of the DNA, great care was taken during extraction and PCR (sectioning the blocks to several small groups at different times, using new surgical blade for each sample and filter tips during extraction and PCR).

The adequacy of the DNA in each specimen for PCR amplification was determined by detection of a 110- or 268-base pair fragment of the β-globin gene using the PC03/PC04 and GH20/PC04 primer set, respectively [16].

For detection of HPV genome, nested PCR were performed using MY09-MY11 as outer and GP5+ to GP6+ as inner primers [14,17]. For genotyping of HPV, the positive PCR products were extracted from the agarose gel and the extracted DNA was analysed by sequencing. Sequence analysis was carried out using the ABI 377 DNA sequencer (PE Biosystems).

TP53 codon polymorphism was detected by allele specific amplification [18]. Ten microliters of the amplification products were analysed by electrophoresis on 2% agarose gels and ethidium bromide staining. The Arg and Pro alleles gave a 141 and 177 bp PCR products, respectively.

2.3. Data processing

Data were processed by BLAST, SPSS statistical software program version 11.5.0. The correlations were subjected to χ^2 (Pearson chi-square) and Fisher's exact test. Odds ratios and logistic regression were also calculated. Statistical significance was set as a *P*-value less than 0.05.

3. Results

The characteristics of study subjects including age, gender, smoking status, HPV DNA, TP53 codon 72 variants and lung cancer types are shown in Table 1. A total of 233 individuals, including 141 lung cancer patients (27 females and 114 males) and 92 non-cancer controls (24 females and 68 males), were recruited into this study (Table 1). Statistical difference was observed in the smoking status ($P \leq 0.0001$) and TP53 codon 72 variants ($P = 0.004$) between these two groups (Table 1). The mean ages of both groups were 66.32 ± 11.34 (S.D.) and 54.39 ± 17.454 (S.D.) years, respectively (Table 1). The gender distribution in case group was comparable with that for the control group ($P = 0.211$, Table 1). The most abundant type of cancer determined histologically was NSCLC (87.2%) and among NSCLC type, squamous cell cancer is predominant, followed by adenocarcinoma and large cell carcinoma (Table 1).

In the healthy control group, the allele frequency for Pro was 0.376, whereas in the lung cancer group was 0.326. The homozygote genotype Pro/Pro was 2.73 times more frequent in controls than in lung cancer subjects (21.3% versus 7.8%, Table 1), but the frequency of Arg/Arg genotype was relatively similar in both groups (42.6% for cases versus 46.1% for controls, Table 1) and the genotype Arg/Pro was 1.5 times more frequent in the cases than the controls (49.6% versus 32.6%, Table 1).

Table 1 The clinical characteristics and HPV status of lung cancer patients and non-cancer controls

Parameter	Cases[a] ($N=141$)	Controls[a] ($N=92$)	P-value
Age (year ± S.D.)	66.32 ± 11.34	54.39 ± 17.454	≤0.0001 (t-test)
Gender			
Female	27 (19.1)[b]	24 (26.1)	0.211
Male	114 (80.9)	68 (73.9)	
Smoking status			
Active	108 (76.6)	42 (45.7)	≤0.0001
Passive	8 (5.7)	7 (7.6)	
Non	25 (17.7)	43 (46.7)	
p53 codon 72 variants			
Arg/Arg	55 (42.6)	41 (46.1)	0.004
Arg/Pro	64 (49.6)	29 (32.6)	
Pro/Pro	10 (7.8)	19 (21.3)	
HPV			
Positive	33 (25.6)	8 (9.0)	0.002
Negative	96 (74.4)	81 (91.0)	
HPV type (%within subject)			
High risk	24 (18.6)	3 (3.4)	
Intermediate risk	4 (3.1)	0 (0)	0.001
Low risk	5 (3.9)	5 (5.6)	
Negative	96 (74.4)	81 (91)	
Histological type			
NSCLC (%within tumour type)	123 (87.2)		
AdC	16		
SCC	104		
LCC	3		
SCLC (%within tumour type)	18 (12.8)		

[a] Some of the subjects have been considered as missing value after quality examination of nucleic acid extraction.
[b] Number in parentheses are percentages.

As indicated in our previous study [14], prevalence of HPV DNA was higher in lung cancer patients than in non-cancer controls (25.6% versus 9.0%, $P=0.002$; Table 1).

In Tables 2 and 3, genotype frequency of TP53 codon 72 and HPV DNA detection rate are stratified by sex and smoking status. In lung carcinomas, genotype Arg/Arg is visible to be related to HPV infection ($P=0.003$, Table 2). As shown in Table 3, the significant differences were observed among TP53 genotypes considering gender, smoking status and HPV type. Regarding to HPV type, Arg allele is statistically related to high risk HPV types (Arg/Arg, $P=0.015$ and Arg/Pro, $P=0.04$, Table 3).

No significant difference was observed between two major tumour type considering gender, smoking status, HPV and TP53 codon 72 genotypes, but discrepancies were observed between SCC and adenocarcinomas patients (Table 4):

(a) Adenocarcinomas female patients are more prevalent than SCC female patients (37.5% versus 13.5%, $P=0.027$).
(b) SCC type lung cancer patients are mostly active smokers (85.6%) whereas the halves of adenocarcinomas patients are non-smokers ($P\leq 0.0001$).
(c) Arg allele is statistically related to SCC type lung cancer (SCC 0.6785 versus AdC 0.429, $P\leq 0.0001$) whereas Pro allele is visible to be related to adenocarcinoma lung cancer (SCC 0.3215 versus AdC 0.571, $P\leq 0.0001$).

The ORs of several parameters of lung cancer patients are shown in Table 5. Due to incomparability of the age means of case and control, age parameter is then adjusted in all parameter. The HPV infection had an AOR of 2.580 (95% CI 1.166-5.708; $P=0.010$). Meanwhile infection of high risk HPV types had a higher AOR of lung cancer incidence as 4.140 (95% CI 1.240—13.809; $P=0.010$), compared with 2.181 (95% CI 1.114—4.270; $P=0.021$) of smoking status. HPV infection was increased the risk of lung cancer in males and was not significant in females (female AOR 2.432, 95% CI 0.543—10.890, $P=0.156$ versus male AOR 2.778, 95% CI 1.147—6.732, $P=0.011$).

According to the smoking status, this significance was observed only in male subjects (male AOR 2.794, 95% CI

Table 2 HPV status according to clinical characteristics of lung cancer patients and non-cancer controls

Variable	Cases		Control		*P*-value
	HPV positive (%)	Number of subjects	HPV positive (%)	Number of subjects	
Age					
≤60	12 (40.0)	30	0	52	≤0.0001
>60	21 (21.9)	96	8 (21.6)	38	1.000
P-value	0.490		0.001		
Gender					
Female	6 (24)	25	2 (8.7)	23	0.249
Male	27 (26)	104	6 (9.1)	66	0.007
P-value	0.840		1.000		
Smoking status					
Active	27 (26.7)	101	4 (9.5)	42	0.023
Passive	1 (14.3)	7	2 (28.6)	7	1.000
Non	5 (23.8)	21	2 (5.0)	40	0.042
P-value	0.750		0.155		
p53 codon 72 variants					
Arg/Arg	22 (40.0)	55	5 (12.2)	41	0.003
Arg/Pro	10 (15.6)	64	2 (6.9)	29	0.329
Pro/Pro	1 (10.0)	10	1 (5.3)	19	1.000
P-value	0.005		0.707		

1.055—7.394; $P = 0.039$ versus female AOR 1.974, 95% CI 0.572—6.805; $P = 0.282$). Arg allele had also an AOR of 3.349 (95% CI 1.334—8.410; $P = 0.007$). As shown in Table 5, when the significant parameters are combined, an increased risk is seen, comparing with the risk of each parameter alone. The data (not shown) reveal that the Arg/Arg-harbored and active smoker males had an OR 17.5 (95% CI 1.551—197.435; $P = 0.041$) of developing SCC lung cancer in north of Iran, in contrast the Pro/Pro harbored-patients are at a higher risk of adenocarcinoma development (AOR 19.244, $P \leq 0.0001$).

To avoid the effect of multiple parameters, we ran a logistic regression model. Data shown that Arg allele (OR 4.576, 95% CI 1.636—12.803; $P = 0.004$), active smoking (OR 4.563, 95% CI 1.691—12.310; $P = 0.003$) and HPV infection (OR 3.030, 95% CI 1.216—7.546; $P = 0.017$) are the significant risk factors in lung carcinogenesis.

4. Discussion

Unquestionably, cigarette smoking does cause many health problems, but certainly it cannot be the only causative factor in lung cancer. Thus, factors other than smoking may also have an impact as risk factors for lung cancer [19,20].

In our previous study [14] we showed that HPV infection may be associated with the development of lung cancer in Mazandaran, north part of Iran, and the low prevalence of HPV infection in lung cancer, as compared with genital

Table 3 TP53 genetic polymorphism according to clinical characteristics of lung cancer patients and non-cancer controls

Parameter	Cases			Control			*P*-value
	RR	RP	PP	RR	RP	PP	
Gender							
Male	46 (44.3)[a]	51 (49)	7 (6.7)	34 (51.5)	18 (27.3)	14 (21.2)	0.002
Female	9 (28.6)	13 (28.6)	3 (42.9)	7 (30.4)	11 (47.8)	5 (21.7)	0.794
Smoking status							
Active	43 (42.6)	51 (50.5)	7 (6.9)	19 (45.2)	13 (31.0)	10 (23.8)	0.008
Passive	2 (28.6)	2 (28.6)	3 (42.9)	4 (57.1)	2 (28.6)	1 (14.3)	0.790
Non	10 (47.6)	11 (52.4)	0	18 (45)	14 (35)	8 (20)	0.073
HPV type							
High risk	13 (23.6)	10 (15.6)	1 (10.0)	3 (7.3)	0	0	Arg/Arg; 0.015
Intermediate risk	4 (7.3)	0	0	0	0	0	Arg/Pro; 0.04
Low risk	5 (5.2)	0	0	2 (4.9)	2 (6.9)	1 (5.3)	Pro/Pro; 0.579

[a] Number in parentheses are percentages.

Table 4 Histological type of lung cancer according to clinical characteristics of lung cancer patients

Variable	Major tumour type		Subtype of NSCLC	
	NSCLC	SCLC	SCC	AdC
Gender				
Female	21 (17.1)[a]	6 (33.3)	14 (13.5)	6 (37.5)
Male	102 (82.9)	12 (66.7)	90 (86.5)	10 (62.5)
P-value	0.114		0.027	
HPV				
Positive	28 (25)	5 (29.4)	24 (25.3)	3 (21.4)
Negative	84 (75)	12 (70.6)	71 (74.7)	11 (78.6)
P-value	0.767		1.000	
Smoking status				
Active	97 (78.9)	11 (61.1)	89 (85.6)	6 (37.5)
Passive	6 (4.9)	2 (11.1)	4 (3.8)	2 (12.5)
Non	20 (16.3)	5 (27.8)	11 (10.6)	8 (50.0)
P-value	0.174		≤0.0001	
p53 codon 72 variants				
Arg/Arg	44 (39.3)	11 (64.7)	37 (38.9)	4 (28.6)
Arg/Pro	59 (52.7)	5 (29.4)	55 (57.9)	4 (28.6)
Pro/Pro	9 (8.0)	1 (5.9)	3 (3.2)	6 (42.8)
P-value	0.140		≤0.0001	

[a] Number in parentheses are percentages.

carcinoma, is leaving room for agents other than HPV infection. In this study, we have evaluated the association between the lung cancer and HPV infection and the genotype at codon 72 of the TP53 gene in north part of Iran.

Taking into account only the healthy control group, the allele Pro frequency was 0.376. Thus the Iranian people (Mazamdaran province) represent a Pro allele frequency higher than American, Mexican-American, Polish, Czech, Spanish, German and English people, nearly equal to

Table 5 The lung cancer risk estimation according to clinical characteristics, HPV and TP53 codon 72 polymorphism status in Iran, Mazandaran province

Variable	OR (95% CI)	AOR (95% CI) (age-adjusted)
Gender		
Female	0.671 (0.359—1.256) ($P = 0.211$)	0.933 (0.470—1.851) ($P = 0.842$)
Male	1.490 (0.796—2.788) ($P = 0.211$)	1.093 (0.539—2.218) ($P = 0.802$)
Smoking status		
Active	4.072 (2.245—7.385) ($P < 0.0001$)	2.181 (1.114—4.270) ($P = 0.021$)
Passive	0.730 (0.256—2.088) ($P = 0.556$)	0.804 (0.293—2.184) ($P = 0.617$)
Smoking status		
Male	5.963 (2.559—13.896) ($P \leq 0.0001$)	2.794 (1.055—7.394) ($P = 0.039$)
Female	3.438 (0.919 —12.865) ($P = 0.060$)	1.974 (0.572—6.805) ($P = 0.282$)
HPV infection	3.480 (1.522—7.958) ($P = 0.002$)	2.580 (1.166—5.708) ($P = 0.010$)
Infection in female	3.316 (0.596—18.851) ($P = 0.249$)	2.432 (0.543—10.890) ($P = 0.156$)
Infection in male	3.506 (1.360—9.038) ($P = 0.007$)	2.778 (1.147—6.732) ($P = 0.011$)
HPV high risk type	6.085 (1.776—20.851) ($P = 0.001$)	4.140 (1.240—13.809) ($P = 0.010$)
p53 codon 72 variants		
Arg	3.230 (1.422—7.339) ($P = 0.004$)	3.349 (1.334—8.410) ($P = 0.007$)
Pro	1.149 (0.667—1.979) ($P = 0.616$)	1.030 (0.576—1.843) ($P = 0.921$)
[HPV+] [Arg/Arg]	4.80 (1.630—14.132) ($P = 0.003$)	3.692 (1.366—9.977) ($P = 0.004$)
[HPV+] [active smoker]	3.466 (1.130—10.629) ($P = 0.023$)	3.390 (1.162—9.895) ($P = 0.015$)
[HPV+] [Arg/Arg] [active smoker]	3.840 (0.972—15.171) ($P = 0.046$)	3.744 (1.069—13.109) ($P = 0.023$)

Korean, French and Italian people and lower than Japanese, Taiwanese and Afro-American people.

This study reveals that the Arg allele of TP53 polymorphism was associated with an increased risk of lung cancer development (Tables 1 and 5; AOR 3.349, $P = 0.007$). Studies of polymorphisms in TP53 are currently less well developed. In most of same type studies, the allelic frequencies of the proline variant do not differ between lung cancer cases and controls. However, the proline variant was the major allele in African-Americans compared to Japanese, Caucasians, and Mexican-Americans where the arginine allele was found to be most prevalent [5,7—10,12,13,21,22]. Among Japanese, Kawajiri et al. [8] considered allelic distribution as it pertained to the Hardy—Weinberg equilibrium. Although the arginine/proline allelic frequencies were not different between cases and controls (arginine allele 0.653 in controls, 0.645 in cases), among controls the allelic distribution was in Hardy—Weinberg equilibrium whereas in cases it was not. In cases there was an underrepresentation of heterozygotes and an overrepresentation of proline homozygotes ($P < 0.005$). In contrast, Murata et al. [9] examined the relationship between the codon 72 polymorphism, lung cancer, and tobacco smoking in a Japanese population but reported an association of TP53 with tendency to smoke tobacco, where the arginine allele was elevated in nonsmoking cancer patients (chi-square = 13.5, $P < 0.001$). Jin et al. [10] detected elevated lung cancer risk associated with inheritance of the TP53 proline allele in a subset of African-American lung cancer cases diagnosed prior to the age of 53 years. Overall, however, Jin et al. found no cancer risk associated with inheritance of the TP53 proline allele [10]. To-Figueras et al. [21] investigated this polymorphism in relation to histologic type of lung cancer in a Caucasian population in Catalonia. In this population no association of the TP53, codon 72 genotype, was observed [21]. Taiwanese and Chilean studies showed that the Pro allele has been related to an increase in lung cancer risk in their population [5,22].

Cigarette smoking is less strongly related to lung adenocarcinoma risk than it is to SCC risk [23]. Nonetheless, although most patients with lung carcinoma have been heavy smokers at some point during their lifetimes, the prevalence of SCC has diminished steadily with the increasing prevalence of adenocarcinoma. Since the early 1980s, adenocarcinoma has been the most common lung malignancy found in North America; in contrast, in Europe, SCC remains the most common subtype of lung disease [24]. Overall, increasing lung adenocarcinoma incidence has been noted among nonsmokers, especially in women, in the U.S. as well as in Asian countries (e.g., Taiwan, Japan) [25]. No lung cancer statistics have been available with which to estimate the histological lung cancer type in Iran. This study shows that SCC type lung cancer is the most frequent subtype of NSCLC in Mazandaran, but adenocarcinoma female patients are more prevalent than SCC type-female patients as it is seen in most part of the world (Tables 1 and 4). Adenocarcinomas lung cancer is more related to non-smoking status and Pro allele, in contrast most of the SCC lung cancer patients are smoker and harbor Arg allele (Table 4, $P \leq 0.0001$).

The oncogenic HPV E6 proteins have been shown to preferentially target p53Arg over p53Pro for ubiquitin-mediated degradation [18], and this manifests itself in an overrepresentation of p53Arg alleles in patients with HPV-associated tumours. This research emphasizes the relation between HPV and Arg/Arg genotype and overall between HPV types and Arg allele (Tables 2 and 3).

Although human papillomavirus has been identified in pulmonary squamous epithelial samples [26] (the high prevalence of HPV infection, 69—73%, in SCC in countries such as Japan, China, Taiwan, Greece and Finland), HPV infection has not been thoroughly assessed as a potential risk factor for the development of pulmonary adenocarcinoma. Recent studies involving Taiwanese patients [27,28] have demonstrated a possible association between HPV infection and pulmonary adenocarcinoma. Among patients who developed lung adenocarcinoma, the percentage with pulmonary HPV infection ranged from 9% to 42% in Asian studies and 0% to 36% in western world studies [25]. In this study, there is no difference between SCC and AdC in detection of HPV and HPV plays a role as a risk factor in the development of pulmonary adenocarcinoma and squamous cell carcinoma (Table 4).

Studies have demonstrated associations between lung carcinoma and a variety of risk factors, including tobacco smoke (active and environmental), other indoor pollutions (cooking oil vapors, coal burning, and fungus spores), diet, genetic factors and infections [19,25]. Tobacco smoking is an iatrogenic agent for production of squamo—columnar junctions (SCJs) in respiratory tract, which is the preferable site of HPV entering [26]. In parallel of some studies which show the synergism of HPV infection and tobacco smoking in lung cancer [29], our study indicates that HPV infection is related to active smoking status (Tables 2 and 5). Furthermore Arg allele is visible to be related to tobacco smoking. As expected, the OR for lung cancer increased with tobacco smoking and HPV infection for Arg/Arg genotype (Table 5). Smoking males harboring Arg/Arg genotype encounter a high risk of developing SCC type lung cancer (OR 17.5, $P = 0.041$). Moreover the Pro/Pro harbored-patients are at a high risk of adenocarcinoma development (AOR 19.244, $P \leq 0.0001$).

No HPV infection and tobacco smoking were observed as a risk factor in under-studied females in spite of what was seen in males in this research (Tables 2 and 5). One of the possible contributing explanations is that women tend to underreport their smoking history, resulting in an apparent increase in lung cancer incidence among nonsmokers. Further studies are needed on lifestyle and other environmental factors in Iranian females to reach a better understanding.

In conclusion, according to the regression analysis Arg allele, active smoking and HPV infection are, respectively, the most important risk factors in lung cancer development in the north part of Iran, Mazandaran province.

Conflict of interest

None.

Acknowledgments

This study was financially supported by the Institute of Public Health Research, Academic Pivot for Education and Research, Tehran University of Medical Sciences.

References

[1] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74—108.

[2] Ahuja HG, Testa MP, Cline MJ. Variation in the protein coding region of the human p53 gene. Oncogene 1990;5:1409—10.

[3] Felix CA, Brown DL, Mitsudomi T, Ikagaki N, Wong A, Wasserman R, et al. Polymorphism at codon 36 of the p53 gene. Oncogene 1994;9:327—8.

[4] Felley-Bosco E, Weston A, Cawley HM, Bennet WP, Harris CC. Functional studies of a germ-line polymorphism at codon-47 within the p53 gene. Am J Hum Genet 1993;53:752—9.

[5] Wang YC, Chen CY, Chen SK, Chang YY, Lin P. p53 codon 72 polymorphism in Taiwanese lung cancer patients: association with lung cancer susceptibility and prognosis. Clin Cancer Res 1999;5:129—34.

[6] Malkin D, Jolly KW, Barbier N, Look AT, Friend SH, Gebhardt MC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasm. Science 1990;250:1233—8.

[7] Weston A, Perrin LS, Forrester K, Hoover RN, Trump BF, Harris CC, et al. Allelic frequency of a *p53* polymorphism in human lung cancer. Cancer Epidemiol Biomark Prev 1992;1:481—3.

[8] Kawajiri K, Nakachi K, Imai K, Watanabe J, Hayashi SI. Germ line polymorphisms of *p53* and *CYP1A1* genes involved in human lung cancer. Carcinogenesis 1993;14:1085—9.

[9] Murata M, Tagawa M, Kimura M, Kimura H, Watanabe S, Saisho H. Analysis of a germ line polymorphism of the *p53* gene in lung cancer patients; discrete results with smoking history. Carcinogenesis 1996;17:261—4.

[10] Jin X, Wu X, Roth JA, Amos CI, King TM, Branch C, et al. Higher lung cancer risk for younger African-Americans with the Pro/Pro *p53* genotype. Carcinogenesis 1995;16:2205—8.

[11] Beckman G, Birgander R, Själander A, Saha N, Holmberg PA, Kivela A, et al. Is p53 polymorphism maintained by natural selection? Hum Hered 1994;44:266—70.

[12] Weston A, Ling-Cawley HM, Caporaso NE, Bowman ED, Hoover RN, Trump BF, et al. Determination of the allelic frequencies of an L-*myc* and a *p53* polymorphism in human lung cancer. Carcinogenesis 1994;15:583—7.

[13] Birgander R, Själander A, Rannug A, Alexandrie AK, Sundberg MI, Seidegard J, et al. *P53* polymorphisms and haplotypes in lung cancer. Carcinogenesis 1995;16:2233—6.

[14] Nadji SA, Mokhtari-Azad T, Mahmoodi M, Yahyapour Y, Naghshvar F, Torabizadeh J, et al. Relationship between lung cancer and human papillomavirus in north of Iran, Mazandaran Province. Cancer letters 2006; in press.

[15] Impraim CC, Saiki RK, Erlich HA, Teplitz RL. Analysis of DNA extracted from formalin-fixed, paraffin-embedded tissues by enzymatic amplification and hybridization with sequence-specific oligonucleopeptides. Biochem Biophys Res Commun 1987;142:710—6.

[16] Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA. Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. Nature 1986;324:163—6.

[17] Sotlar K, Diemer D, Dethleffs A, Hack Y, Stubner A, Vollmer N, et al. Detection and typing of human papillomavirus by E6 nested multiplex PCR. J Clin Microbiol 2004;42:3176—84.

[18] Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, et al. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature 1998;393:229—34.

[19] Lam WK. Lung cancer in Asian women—the environment and genes. Respirology 2005;10:408—17.

[20] Alberg AJ, Samet JM. Epidemiology of lung cancer. Chest 2003;123:21S—49S.

[21] To-Figueras J, Gené M, Gómez-Catalàn, Firvida J, Fuentes M, Rodamilans M, et al. Glutathione *S*-transferase M1 and codon 72 p53 polymorphism in a northwestern Mediterranean population and their relation to lung cancer susceptibility. Cancer Epidemiol Biomarkers Prev 1996;5:337—42.

[22] Irarraĭzabal CE, Rojas C, Aracena R, Marquez C, Gil L. Chilean pilot study on the risk of lung cancer associated with codon 72 polymorphism in the gene of protein p53. Toxicol Lett 2003;144:69—76.

[23] Devesa SS, Shaw GL, Blot WJ. Changing patterns of lung cancer by histologic type. Cancer Epidemiol Biomarkers Prev 1991;1:29—34.

[24] Travis WD, Travis LB, Devesa SS. Lung cancer. Cancer 1995;75:191—202.

[25] Chen YC, Chen JH, Richard K, Chen PY, Christiani DC. Lung adenocarcinoma and human papillomavirus infection. Cancer 2004;101:1428—36.

[26] Syrjänen KJ. HPV infections and lung cancer. J Clin Pathol 2002;55:885—91.

[27] Chiou HL, Wu MF, Liaw YC, Cheng YW, Wong RH, Chen CY, et al. The presence of human papillomavirus type 16/18 DNA in blood circulation may act as a risk marker of lung cancer in Taiwan. Cancer 2003;97:1558—63.

[28] Cheng YW, Chiou HL, Sheu GT, Hsieh LL, Chen JT, Chen CY, et al. The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking Taiwanese women. Cancer Res 2001;61:2799—803.

[29] Chang KW, Chang CS, Lai KS, Chou MJ, Choo KB. High prevalence of human papillomavirus infection and possible association with betel quid chewing and smoking in oral epidermoid carcinomas in Taiwan. J Med Virol 1989;28:57—61.