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Relationship between lung cancer and human papillomavirus in north of Iran, Mazandaran province

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Abstract

Lung cancer is a major health problem and the leading cause of cancer deaths in the world. The pathogenesis of lung cancer is complex, and is believed to be due to the interaction between environmental and genetic factors. Various evidences show that HPV might be involved in bronchial carcinogenesis. In this study, 141 lung cancer patients and 92 non-cancer control subjects were enrolled to examine whether HPV DNA existed in lung tumor and normal tissues in Mazandaran, north part of Iran by nested PCR. Our data showed that 33 of 129 lung tumors had HPV DNA compared with 8 of 90 non-cancer control subjects (25.6% vs. 9.0%, $P = 0.002$). The infection of HPV had an OR of 3.48 (95% CI 1.522–7.958; $P = 0.002$). Meanwhile infection of high risk HPV types (16 and 18) had a significantly high OR of lung cancer incidence as 8.00 (95% CI 1.425–44.920; $P = 0.021$) compared with 4.423 (95% CI 2.407–8.126; $P \leqslant 0.0001$) of smoking status. This result suggests that HPV infection is associated with lung cancer development in Mazandaran, Iran.

Keywords: Lung cancer; Human papillomavirus (HPV); HPV type; Iran

1. Introduction

Lung cancer has been the most common cancer in the world since 1985, and by 2002, there were representing 12.4% of all new cancers. It was also the most common cause of death from cancer, with 17.6% of deaths the world total [1]. In the USA, it has been the leading cause of cancer deaths in men in the past five decades, and since 1987 has overtaken breast cancer as the leading cause of

Abbreviations: HPV, human papillomavirus; PCR, polymerase chain reaction; SD, standard deviation; OR, odds ratio; CI, confidence interval; LCLC, large cell lung carcinoma; AdC, adenocarcinoma; SQC, squamous cell carcinoma; LCC, large cell carcinoma; SCLC, small-cell lung carcinoma.

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cancer death in women. It currently accounts for 27% of all cancer deaths in women each year, which is more than cancer deaths due to breast, colon and rectum combined [2]. In Europe, lung cancer is also a leading cause of cancer mortality, accounting for 28% of cancer deaths in men and 10% of cancer deaths in women [3].

For almost 30 years no population-based cancer statistics have been available with which to estimate the cancer burden in Iran. In 2002 and 2003, two separate reports of population-based cancer registries were published from Iran and suggest that the incidence of lung cancer is very low [4]. According to the Annual Reports of Health Research Station of Babol city, School of Public Health, Tehran University of Medical Sciences, lung cancer was representing 2.8–4.6% of all new cancers in Mazandaran province from 1999 to 2004 (data is not published).

Human lung cancers are classified into two major types, small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), the latter consisting of several types [5].

The pathogenesis of lung cancer is complex, and is believed to be due to the interaction between environmental and genetic factors. Environmental tobacco smoke, cooking oil vapors, indoor smoky coal burning and infection with tuberculosis and human papillomavirus are risk factors in different populations. The interactions between different genes and environmental factors are beginning to be appreciated and should be further elucidated [6]. Although tobacco smoking is the most important environmental risk factor for the development of lung cancer but high rate of lung cancer despite a low prevalence of smoking in some population have provided useful information on risk factors other than smoking [7]. This is emphasizing the importance of the search for additional aetiological and risk factors.

HPV has been shown to be implicated in human neoplasm including uterine cervix, vulva, skin, esophagus and head and neck [8]. According to the present data, HPV is most commonly associated with the development of cervical carcinomas and HPV 16/18 are the types most frequently detected in high-grade squamous intraepithelial lesions and invasive carcinomas.

Clearly, the epidemiological and morphological observations, the detection of HPV DNA in lung cancer and in vitro studies are in agreement with the concept that HPV might be involved in bronchial carcinogenesis [9]. The detection rates of HPV in lung carcinomas are subject to wide variations. In a review of 85 studies [9] and some others [10,11] recording about 2739 cases described a detection rate of 22.16%.

In this study, we tried to address the question of a viral aetiology of lung cancers by investigating of HPV genome in a series of 233 cases of lung carcinomas and controls with PCR and sequencing for the presence of different genotypes of HPV in northern province of Iran, Mazandaran.

2. Materials and methods

2.1. Tissue samples

A total of 233 blocks of paraffin-embedded tissue including 144 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 non-cancer patients with different lung diseases, including pneumothorax, cryptococcal and Hydatic cyst infection and fibrosis served as control subjects.

2.2. Nested PCR

Genomic DNA was prepared from a tissue section and isolated by conventional phenol–chloroform extraction and ethanol precipitation and was finally dissolved in 80 μl of sterile distilled water. To avoid contamination during extraction of the DNA, great care was taken. The blocks were sectioned to several small groups at different time over a period of one week. New surgical blade was used for each sample. In addition to monitor the contamination, a few negative controls (water samples) were used in each round of extraction. Also the filter tips were used both during extraction and PCR procedures.

To exclude false-negative results, 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 after amplification using the PC03/PC04 and GH20/PC04 primer set, respectively [12].

For detection of HPV genome, nested PCR were performed using MY09-MY11 as outer and GP5+-GP6+ as inner primers [13]. In the first round PCR with primers MY09-MY11 was performed in a final volume of 50 μl. Each PCR mixture contained 50 mM KCl, 10 mM Tris–HCl (pH 8.5), 4 mM $MgCl_2$, a 200 μM concentration of each dNTP, 2 U of FastStart DNA Polymerase (Roche, Germany) and 1 μM of primers MY09 and MY11. Amplification were performed with the following cycling profile: FastStart DNA

polymerase activation was performed by incubation at 94 °C for 10 min followed by 40 cycles of 1-min denaturation at 94 °C, 1-min annealing at 55 °C and 1-min elongation at 72 °C in a PCR cycler (Gradient type model PC-818-02, Astec Co., Ltd., Japan). The last cycle was followed by a final 5-min extension step at 72 °C.

In the second round PCR with primers GP5+-GP6+, 5 μl of MY09-MY11 PCR product was used as template. The nested PCR was performed in a final volume of 50 μl. Each PCR mixture contained 50 mM KCl, 10 mM Tris–HCl (pH 8.5), 2.5 mM $MgCl_2$, a 200 μM concentration of each dNTP, 2 U of FastStart DNA Polymerase (Roche, Germany) and 1 μM of primers GP5+ and GP6+. The cycling conditions were as follows: a 10 min FastStart DNA polymerase activation step at 94 °C was followed by 40 cycles of 1-min denaturation at 94 °C, 1.5-min annealing at 40 °C and 1.5-min elongation at 72 °C. The last cycle was followed by a final 5-min extension step at 72 °C. Ten microliters of the amplification products were analyzed by electrophoresis on 2% agarose gels and ethidium bromide staining.

2.3. Sequencing of PCR products

PCR products were purified with a gel extraction kit (Qiagen) and sequenced by the dye-deoxy terminator method on a 377 ABI Prism Sequencer (PE Applied Biosystems).

2.4. 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. Statistical significance was set as a P value less than 0.05.

3. Results

3.1. Descriptive data

A total of 233 individuals, including 141 lung cancer patients and 92 non-cancer controls, were recruited into this study. The mean ages of both groups were 66.32 ± 11.34 (SD) and 54.39 ± 17.454 (SD) years, respectively. The lung carcinoma samples were related to 27 female and 114 male patients and the control samples were related to 24 female and 68 male patients. The gender distribution in case group was comparable with that for the control group ($P = 0.211$). Statistical difference was observed in the parameter of smoking status between these two groups (76.6% in cases vs. 45.7% in controls, $P \leqslant 0.0001$). The characteristics of study subjects including age, gender and smoking status, the detection frequency of HPV DNA and lung cancer types are shown in Table 1.

3.2. Prevalence of HPV DNA was higher in lung cancer patients than of non-cancer controls

From the detection of HPV DNA by nested PCR, it was shown that the prevalence rates of HPV in lung cancer patients were significantly higher than those in the control group (25.6% vs. 9.0%, $P = 0.002$; Table 1).

As further shown in Table 2, in lung carcinomas significant difference was not observed between HPV presence and age groups, gender, smoking status and tumor types. Also no significant relationship was observed between LCLC types ($P = 1.0$).

Regarding to HPV types, the prevalence of high risk HPV types (HPV 16 and 18) was higher than that of other HPV types in cases (72.7%) but in controls, the low risk HPV type are prevalent (62.5%). This discrimination was statistically significant ($P = 0.036$).

3.3. Presence of HPV DNA in the lung cancer patients had a significantly higher OR of lung cancer incidence

Based on the significant prevalence of HPV in lung cancer, the ORs of HPV presence and several other parameters of lung cancer patients were statistically analyzed (Table 4).

The infection of HPV had an OR of 3.48 (95% CI 1.522–7.958; $P = 0.002$). Meanwhile infection of high risk HPV types (16 and 18) had a significantly high OR of lung cancer incidence as 8.00 (95% CI 1.425–44.920; $P = 0.021$) compared with 4.423 (95% CI 2.407–8.126; $P \leqslant 0.0001$) of smoking status. According to the smoking status, this significance was observed only in male subjects (male OR 6.20, 95% CI 2.74–15.041; $P \leqslant 0.0001$ vs. female OR 6.25, 95% CI 0.662–59.027; $P = 0.184$).

4. Discussion

The possibility that HPV, as co-carcinogen, might contribute to development of lung cancer, is an important one. 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 [14,15].

For detection of HPV DNA, paraffin-embedded tissues were analyzed by nested PCR. As shown in Table 1, the detection frequency of HPV DNA in lung cancer patients (25.6%) was significantly higher than that of non-cancer (control) patients (9.0%, $P = 0.002$). This indicates the possible role of HPV infection in lung cancer in the north part of Iran.

When the study subjects were stratified by gender, age, smoking status and the lung cancer types

Table 1
The characteristics of study subjects and prevalence of HPV DNA and smoking status in lung cancer patients and non-cancer controls

Parameter	Cases[a] ($N = 141$)	Controls[a] ($N = 92$)	P value
Age (year ± SD)	66.32 ± 11.34	54.39 ± 17.454	–
Gender			0.211
Female	27 (19.1)[b]	24 (26.1)	
Male	114 (80.9)	68 (73.9)	
Smoking status			⩽0.0001
Active	108 (76.6)	42 (45.7)	
Passive	8 (5.7)	7 (7.6)	
Non	25 (17.7)	43 (46.7)	
HPV			0.002
Positive	33 (25.6)	8 (9.0)	
Negative	96 (74.4)	81 (91.0)	
HPV type (% within type)			0.036
High risk	24 (72.7)	3 (37.5)	
Intermediate risk	4 (12.1)	0	
Low risk	5 (15.2)	5 (62.5)	
Tumor type			
LCLC (% within tumor type)	123 (87.2)		
AdC	16		
SQC	104	–	–
LCC	3		
SCLC (% within tumor 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.

Table 2
Relationships between the presence of HPV DNA in the study subjects and characteristic parameters

Variable	Cases		Control	
	HPV positive (%)	Number of subjects	HPV positive (%)	Number of subjects
Age				
⩽60	5(41.6)	12	0	40
>60	28(24.6)	114	8(16.3)	49
P value	0.297		0.007	
Gender				
Female	6(24)	25	2(8.7)	23
Male	27(26)	104	6(9.1)	66
P value	0.840		1.000	
Smoking status				
Active	27(26.7)	101	4(9.5)	42
Passive	1(14.3)	7	2(28.6)	7
Non	5(23.8)	21	2(5.0)	40
P value	0.750		0.155	
Tumor type				
LCLC	28(25)	112	–	–
SQC	24(25.3)	95		
AdC	3(21.4)	14		
LCC	1(33.3)	3		
P value	1.00			
SCLC	5(29.4)	17		
P value	0.767			

(Table 2), HPV DNA detection frequency was not statistically different among lung cancer patients, but the difference was observed among controls who were >60 years old ($P = 0.007$).

HPV detection rates in bronchial carcinomas are highly variable in the different studies published from several countries, ranging from 0% to 100% [9–11]. The high prevalence of HPV infection in lung carcinomas in countries such as Japan, China, Taiwan, Greece and Finland reflects the possible association of geographic variability of HPV infection with these lesions. Furthermore the heterogeneity of the results can be reasonably explained by the different sampling modes for the tumor specimens (fresh-frozen or formalin-fixed, paraffin-embedded tissues) and/or different DNA testing methods for HPV genome detection (different hybridization and amplification methods) [9–11].

Like other previous studies [9–11], HPV type-16 was also the predominant type in Iran. So that HPV-16 and -18 (high risk HPV types) were the predominant HPV types, being responsible for 72.7% of the HPV positive cases in lung cancer. Other types detected in bronchial carcinomas were HPV types 6, 11, 26 and 31 (Table 3).

Table 3
HPV types in lung cancer and non-cancer specimens

Study subject	Low risk type		Intermediate risk type		High risk type	
	HPV6	HPV11	HPV26	HPV31	HPV16	HPV18
Lung cancer (%)	**3 (9.1)**	**2 (6.1)**	**1 (3.0)**	**3 (9.1)**	**13 (39.4)**	**11 (33.3)**
LCLC						
AdC	1	0	0	0	0	2
SQC	1	1	0	3	12	7
LCC	0	1	0	0	0	0
SCLC	1	0	1	0	1	2
Non-cancer (%)	**2 (25.0)**	**3 (37.5)**	0	0	0	**3 (37.5)**

Table 4
The risk estimation of lung cancer according to age, gender, smoking status, presence of HPV DNA and HPV genotype

Variable	Groups unfavorable/favorable	OR (95% CI)	*P* value
Gender	Male/female	1.490 (0.796–2.788)	0.211
Smoking status (a)	Active/non-smoker	4.423 (2.407–8.126)	$\leqslant$0.0001
Smoking status (b)	Passive/non-smoker	1.966 (0.636–6.072)	0.235
Smoking status (c)	Active/non-smoker		
Female		6.25 (0.662–59.027)	0.184
Male		6.20 (2.74–15.041)	$\leqslant$0.0001
HPV	Positive/negative	3.480 (1.522–7.958)	0.002
HPV genotype(a)	High/low risk	8.00 (1.425–44.920)	0.021
HPV genotype(b)	Intermediate/low risk	0.556 (0.310–0.997)	0.221

The risk estimation of lung cancer according to age, gender, smoking status, presence of HPV DNA and HPV type was statistically analyzed (Table 4).

These results suggest that HPV infection, especially high risk HPV infection is strongly associated with the lung cancer development (HPV infection OR 3.480, 95% CI 1.522–7.958; $P = 0.184$ and HPV high risk type OR 8.00, 95% CI 1.425–44.920; $P = 0.021$). It is very interesting to observe that the OR of high risk HPV types is higher than that of active smoking. The smoking male but not the smoking female were most at risk of lung cancer in north of Iran (male OR 6.20, 95% CI 2.74–15.041; $P \leqslant 0.0001$ vs. female OR 6.25, 95% CI 0.662–59.027; $P = 0.184$).

In conclusion, 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 carcinoma, is leaving room for agents other than HPV infection.

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