Role of CYP1A1 MspI polymorphism in CYP1A1 gene with susceptibility to lung cancer in Iranian patients

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BACKGROUND: Lung cancer has remained the most prevalent malignancy worldwide. It is the fifth leading cause of cancer death in Iran. Nevertheless, during last few years a gradual permanent increase in its incidence has been reported. Although the crucial role of tobacco smoke in lung cancer initiation has long been established, it is tempting to hypothesize that genetic polymorphisms may contribute to lung cancer predisposition. CYP1A1 gene encodes the main enzyme responsible for metabolic activation of several tobacco carcinogens. CYP1A1 MspI (6235T→C) polymorphism is the most studied variation within the CYP1A1, impacts on the basal levels of metabolism and is believed to be associated with elevated lung cancer risk, mainly in Asian population. METHODS: We investigated the frequency of this genetic variation in Iranian lung cancer patients through a cross-sectional study. 65 lung cancer cases and 80 healthy controls were recruited. RESULTS: The present findings confirmed the low frequency of the variant CYP1A1*2A allele in the control group. A significant increased risk for lung cancer was observed among those who possessed heterozygous (*1/*2A) genotype (Odds ratio = 2.79, 95% CI: 1.01-7.65). Adenocarcinoma was more frequent in non-smoker group (p = 0.00064); however, no significant increased risk was observed for squamous cell carcinoma and small cell carcinoma with respect to smoking. CONCLUSIONS: Heterozygous (*1/*2A) genotype may increase the risk of lung cancer.

KEYWORDS: Cytochrome P450, Lung Cancer, Polycyclic Aromatic Hydrocarbon, RFLP-PCR, Tobacco Smoke, Iran

BACKGROUND

Worldwide, lung cancer accounts for the most common cancer in men and women,[11] causes more than one million deaths each year.[2] However, in Iran it represents lower incidence than expected, since despite of high prevalence of smokers in Iranian male population, lung cancer remains fifth highest malignancy. It appears that its low incidence may be due to under reporting and difficult tissue diagnosis, although recent few studies have shown a gradual continuous increasing in lung cancer occurrence.[3,4]

It has been well established that chronic tobacco smoke inhalation is the most dominant risk factor for lung cancer.[5] Tobacco smoke is a mixture of several thousand chemicals, provides the main source of strong human carcinogens, including polycyclic aromatic hydrocarbons (PAHs), nitrosamines and aromaticamines.[6,7] However, not all the people exposed to tobacco smoke eventually develop the disease, indicating that in addition to various extent of smoking and different exposure to other environmental carcinogens, individual genetic differences in serious metabolizing genes might affect lung cancer carcinogenesis.[8-10]

The enzyme aryl hydrocarbon hydroxylase (AHH) encoded by CYP1A1 gene, is a key member of more significant P450 enzymes, exhibits significant roles in the initial step of metabolic activation of vast numbers of environmental carcinogens.[11] AHH is predominantly expressed in extrahepatic tissues such as the lung, and appears to be more important in the metabolism of PAHs to their respective intermediates. These reactive electrophilic metabolites are high potent to bind to DNA, and lead to adducts formation, the chemical modifications to DNA that can precede mutation events.[12,13]

Several important polymorphisms have been described in the CYP1A1 gene. The first detected variant allele is CYP1A1*2A, which consists of a T to C transition at nucleotide 3801, located in 3’ non-coding region of the gene. This base substitution introduces a new restriction site for MspI endonuclease and hence also known as MspI.[14-16] Some studies that were conducted in Japanese showed a
considerable high frequency for MspI variant allele, whereas inconsistent with Japanese and Asian populations, this polymorphism was reported to be much less frequent in Caucasian populations.\textsuperscript{[13,17,18]}

It has been demonstrated that following exposure to high inducible PAHs, individuals carrying CYP1A1*2A allele could represent enhanced regulation of transcription, or heightened enzymatic activity, although the precise mechanism underling this relation has not been determined.\textsuperscript{[19-21]} However, a number of similar studies investigating the potential association between this genetic variation and lung cancer risk, have reported dissimilar consequences.\textsuperscript{[14,22,23]}

In view of high prevalence of smoking in Iran, and the previously reported ethnic differences in association between CYP1A1 MspI polymorphism and lung cancer risk, the present analysis has been designed to evaluate and compare the frequency of this genetic polymorphism in Iranian lung cancer patients, and to assess the possible association of this polymorphism to lung cancer predisposition.

METHODS

Study population
We have investigated the frequency of this genetic variation in 65 Iranian patients with lung cancer compared to 80 healthy controls through a cross-sectional study (2009-2011).

The lung cancer patients included those who were first diagnosed by standard histopathological procedures at Chronic Respiratory Diseases Research Center, Masih Daneshvari Hospital, Tehran, Iran. To avoid any possible error in selection of primary lung cancer cases, blood collection was always performed under supervision of a medical oncologist.

The control group consisted of 80 cancer free volunteers, unrelated to patients, randomly selected among who refer to the clinics for regular health checks. Prior to blood sample collection a structured questionnaire was completed during a brief face to face interview to obtain demographic characteristics such as age, gender and smoking habits. For patients who were unable to participate in interview, data was collected from a person of subject's intimates or obtained from the medical file, registered by physicians at admission time. The Blood samples collected in tubes containing anticoagulant ethylenediaminetetraacetic acid (EDTA), immediately stored at -20° C until DNA isolation.

Exclusion criteria were as follows: female gender, age, smoking habit, familial history of lung cancer and metastatic activity.

DNA Genotyping
Genomic DNA was extracted from peripheral white blood cells using a salting method. In order to analyze CYP1A1 MspI polymorphism, a distinct region with 373 bp length including the variation site was amplified with PCR technique. Primers used for amplifying were (F: 5'-AGCAGTGAGGCTAGCG-3') and (R: 5'-TAGAGGCGGTAATCAGCA-3'). Each 25 μL PCR reaction mixture consisted of 2 μl of 10 mM of each the forward and reverse primers, 2.5 μL of 10x solution buffer, 0.5 μl of a 10 μM of four mixed dNTPs, 0.75 μl of 50 mM of MgCl2, 0.25 μl of 5u/μl Taq DNA polymerase (Cinnagene, Co., Iran), 2 μl of genomic DNA (80ng/μl) and 15 μl of sterile H2O. The amplification reaction using thermal cycler was carried out as the following conditions: initial denaturation at 95° C in 3 minutes, followed by 35 cycles with melting at 95° C for 30 seconds, annealing at 62° C for 35 seconds, and primer extension at 72° C for 40 seconds. The PCR reaction was completed by a final extension step at 72° C for 10 minutes. The amplified products were then separated by electrophoresis through 1% gel agarose and visualized with ethidium bromide staining.

PCR amplified products were digested to determine genotypes. MspI restriction enzyme (New England Biolabs) was used to distinguish the CYP1A1 MspI polymorphism, at 37° C overnight. MspI enzyme does not digest the wild type CYP1A1*1 allele, and fragments included this allele remain intact (373 bp), while restriction digestion occurred at the presence of polymorphic CYP1A1*2A allele, generates two unequal 127 and 226 bp bands. The restricted products were analyzed by electrophoresis in 1% agarose gel.

Statistical Analysis
The chi-square test was used to compare the distribution of different genotypes and allele frequencies for the polymorphism among both patient and control categories. The same test was also used to determine possible significant association between lung cancer risk and CYP1A1 MspI variant allele. The odd ratios (ORs) and their corresponding 95% confidence interval were calculated to estimate relative relationship between each CYP1A1MspI variant allele and lung cancer. Independent t-test was used to compare numerical variables. Analysis was carried out with SPSS software version 16 (SPSS Inc., Chicago, IL, USA). A probability of P-value less than 0.05 was considered as statistically significant.
RESULTS

The general information of study population is illustrated in details in table 1. The mean age was calculated 53.3 years for the patients and 51.7 for the controls. There were no significant differences in the distribution of sex between cases and controls (p > 0.05). However, a significant difference in smoking status was obtained between two categories. Compared to healthy control group, more smokers were in lung cancer group (p = 0.001). The results of histology findings were as following (AC: Adenocarcinoma SCC: Squamous Cell Carcinoma SCLC: Small Cell Lung Carcinoma):

AC 22 (33.85)  
SCC 9 (13.85)  
SCLC 9 (13.85)  
Other/unknown types 25 (38.45)

Table 2 depicts distribution of different lung cancer histological types. Compared to other types, adenocarcinoma was more frequent in patients, while cases with small cell lung carcinoma and squamous cell carcinoma were much more likely to have a positive smoking history, although no significant difference were observed (p > 0.05).

Detailed analysis of the different CYP1A1 MspI genotypes are presented in table 3. Since there were no individuals with *2A/*2A genotype, who homozygous for CYP1A1*2A variant allele among both cases and controls, *2A/*2A genotype was not shown.

The frequency of *1/*1 and *1/*2A genotypes in the cases were estimated 81.5% and 18.4%, while these genotypes obtained in 95.5% and 7.5% of control subjects, respectively. Thus, the frequency of variant genotypes was low in two groups; however, a significant positive association was observed.
between the *I/*2A genotype and lung cancer risk, (p = 0.047, OR = 2.79 (1.01-7.65)). Nevertheless, distribution of allele frequencies in cases and controls (Table 4) show that there were no significant positive association between the variant CYP1A1*2A allele and lung cancer risk (P = 0.054, OR = 2.61 (0.98-6.91)).

Comparison of CYP1A1 MspI genotypes distribution in lung cancer histological types, as well as interaction of these genotypes and smoking was also assessed separately which in this case, no significant results were found (data not shown).

**DISCUSSION**

The current study was conducted to assess lung cancer susceptibility of Iranian patients for tobacco smoking and the possible role of the most characterized CYP1A1 MspI polymorphism. As expected, the inevitable impact of tobacco smoke inhalation on predisposition to lung cancer was emphasized.

The association of tobacco smoke with different histological types of lung cancer showed a little further risk for small cell lung cancer, although no statistically significant difference was obtained. However, in contrast to our findings, nearly most previous studies suggest a higher risk for squamous cell cancer.

The present findings indicated no clear relationship between different lung cancer types and CYP1A1 MspI polymorphism (data not shown). In this case, different investigations represented dissimilar results in Caucasian populations. However, several reports in Japanese and Chinese, where the mutant allele show higher frequencies, described a considerable relation between CYP1A1 homozygous variant genotype and squamous cell carcinoma. For instance, Song et al.\(^{[21]}\) reported a significant linkage between genetic CYP1A1 MspI and lung squamous cell cancer among Chinese. A significant association between higher risk for the mentioned cancer type and the presence of at least one copy of mutant CYP1A1*2A allele in Asians was also reported.\(^{[12,17]}\)

The pattern of genotype and allelic frequencies obtained in our investigation was almost confirmed the results of previous investigations in Caucasian population, since the variant allele was too low to found any CYP1A1*2A homozygous carriers. Nevertheless, a positive significant association was observed between heterozygous genotype and increased lung cancer risk (p = 0.047, OR = 2.79, 95% CI: 1.01-7.65). This finding differs from the most of reports in Caucasians, since in a few studies a marked relationship have been described. At the end, the limitations of present study could not be ignored. The small sample size might have had an effect on statistical analysis and further investigations on larger population are required to validate these findings. Therefore, in the near future, we will focus our efforts on more expanded population in Iranian lung cancer patients.

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**REFERENCES**


<p>| Table 4. Distribution of CYP1A1 MspI genotypes |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Allele</th>
<th>CYP1A1 MspI</th>
<th>Cases N (f)</th>
<th>Controls N (f)</th>
<th>P-value</th>
<th>OR (CI 95%)</th>
</tr>
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<tr>
<td>CYP1A1*1</td>
<td>118 (0.91)</td>
<td>154 (0.963)</td>
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<tr>
<td>CYP1A1*2A</td>
<td>12 (0.09)</td>
<td>6 (0.037)</td>
<td>0.054</td>
<td>2.61 (0.98-6.91)</td>
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<tr>
<td>Sum</td>
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<td>160</td>
<td></td>
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