GSTM1 Gene Polymorphisms on Lung Cancer Development in the Turkish Population

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Abstract

Glutathione S-transferases (GSTs) play an important role in the detoxification of many xenobiotics involved in the etiology of cancer. A series of studies have suggested that individuals lacking GSTM1 could potentially be at higher risk for lung cancer. In different ethnic groups variations in null allele frequency has been observed. In our study on a Turkish population sample, GSTM1 gene polymorphisms and encoding phase II biotransformation enzymes, were investigated in healthy subjects and in lung cancer patients. DNA samples, extracted from the whole blood were amplified using polymerase chain reaction (PCR) method in all 87 subjects, consisting of 47 previously diagnosed lung cancer patients and 40 healthy controls. The prevalence of GSTM1-null genotype in the lung cancer patients was 51.1%, compared to 57.5% in the control group. No statistical significance related with GSTM1 (0/0) null genotype was found between the control and lung cancer groups (OR =1.30, 95% CI=0.55-3.03, P=0.55). We observed that carrying the GSTM1 genotype is not a risk factor alone for lung cancer.

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Introduction

Lung cancer is the most common malignancy and the leading cause of cancer death in men worldwide and also the second most lethal cancer in women after breast cancer (1,2). Active and passive smoking, various occupational exposures, and carcinogens in heavily polluted air are causally related to lung cancer in humans. These environmental carcinogens are strongly influenced by individual susceptibility factors (3). Only one of ten lifetime smokers develops lung cancers, implying that the differential risk for lung cancer may be explained by genetic susceptibility factors (3). Several of the genes of the enzymes involved in metabolic activation and detoxification of pulmonary carcinogens such as polycyclic aromatic hydrocarbons (PAH) and aromatic amines are known to be polymorphic in humans. Interindividual differences in the ability to activate and detoxify carcinogens are expected to affect the risk of developing lung cancer (4).

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Polymorphisms of the genes encoding phase I and phase II xenobiotic metabolizing enzymes have been shown to be associated with susceptibility to lung cancer in a number of epidemiologic studies (5). However, most of the results presented in this study are limited by lack of adequate statistical power. To overcome this limitation, the International Collaboratiove Study on Genetic Susceptibility to Environmental Carcinogens (GSEC) was begun and is on-going to pool raw data of studies on metabolic genetic polymorphisms and cancer risk (6). Environmental exposures, especially cigarette smoke contains several thousands chemicals, of which about 50 compounds are known carcinogens, including PAHs, aromatic amines and N-nitroso compounds. Some of these compounds are reactive carcinogens, but most are procarcinogens, which need to be activated by phase I enzymes such as those encoded by the CYP super gene family, and converted into reactive carcinogens. All these reactive carcinogens can bind to DNA and form DNA adducts capable of inducing mutation and initiaiting carcinogenesis.

Glutathione S-transferases (GSTs) play an important role in the cellular defence since they are involved in the detoxification of many carcinogens and environmental pollutants and facilitate their excretion and also they are known to have a role in protection against oxidative stress (7,8). Between 38% and 67% of Caucasians carry a deletion in both alleles of the GSTM1 gene resulting in a total absence of GSTM1 enzyme activity (9). Some studies suggest that the GSTM1 null genotype confers an increased risk of lung cancer but this result has not been replicated by others, especially by recent meta and pooled analyses. (6,10-13).

The risk of lung cancer is expected to be monitorized by genetic studies using specific biomarkers. Thus, if we can obtain satisfactory results, meaning that some specific gene polymorphisms have significant roles in susceptibility to lung cancer, it will be possible to inform the patients in potential risk not to be exposed to some environmental factors (smoking, air pollution, food, drug...etc) which are important at least as much as our genetic structure in cancer pathogenesis. Moreover, there will be a great advantage of taking necessary medical precautions in earlier stages.

The aim of the present study was to determine the frequencies of polymorphisims of GSTM1 gene and their association with lung cancer in the Turkish population.

Materials and Methods

The study population consisted of 47 lung cancer patients who attended the Yedikule Teaching Hospital for Chest Diseases and Thoracic Surgery between the years 2000 and 2002. The diagnosis of lung cancer was histologically confirmed in all patients. The mean age of patients were 56±10 years (range, 30-75) Forty-four of the patients were males and 3 were females. Forty-three patients were smokers and 4 patients were nonsmokers. Histological typing of lung cancer was performed according to WHO criteria and confirmed by pathological review.

The control group consisted of 40 healthy individuals. The control group was free of any cancers or chronic diseases. All cases and controls were born in Turkey. The mean age of controls were 35±11 years (range, 20-65). Sex distribution was 25 males and 15 females. Eleven patients were smokers and 29 were nonsmokers. Peripheral blood samples were collected from lung cancer patients and control subjects. DNA was isolated from peripheral blood samples using a DNA isolation kit (Qiagen, Hilden, Germany).

GSTM1 genotyping

DNA samples were amplified with the primers:

5 GAACTCCCTGAAAAGCTAAAGC 3 (forward) and 5-GTTGGGCTCAAATATACGGTGG – 3 (reverse) for GSTM1 which produced a 219 bp product (14). The PCR amplification was carried out using 1 µg DNA in 10 mM Tris-HCL, pH 8.3, 50 mM KCl, 3 mM MgCl2, 0.3 mM deoxyribonucleotide triphosphates (Fermentas), 0.2 µM of each primer and 1.5 U of Taq polymerase (Fermentas) in a total volume of 50 µl. Amplification was performed with initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 61°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min, using a MJ Research PTC160 thermal cycler.

The amplification product (10 µl) was visualized in an ethidium bromide stained 1.5% agarose gel. All genotype determinations were carried out twice in independent experiments and all the inconclusive samples were reanalyzed. The results are shown below (Figure 1).

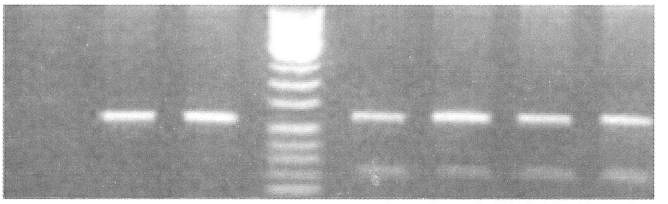

Figure 1. GSTM1 genotyping. (-): water; 1, 2: GSTM1 null genotype (0/0); 3, 4, 5 and 6 GSTM1 +/+ or +/- genotype. M: 100 bp ladder size marker.

Statistical analysis

The statistical analyses were performed using the Statistical Package for Social Sciences Program (SPSS, Version 10). The data were evaluated using the Pearson's test, the χ^2 test and multiple logistic regression tests.

Results

In this study, the GSTM1 genotype of 47 lung cancer patients and 40 controls was determined by PCR analysis. Table 1 presents demographic characteristics of the lung cancer patients and controls.

Table 2 presents frequency of GSTM1 genotypes in lung cancer patients and in the controls. The prevalence of GSTM1 (0/0) genotype in lung cancer patients was 51.1% compared to 57.5% in the control group but the result was not statistically significant (crude OR =1.30, 95% CI=0.55-3.03, P=0.55) (Table 2).

Histopathologically, out of 47 patients, epidermoid carcinoma (EPCA) was detected in 51.1% (n=24), adenocarcinoma (ACA) in 21.3% (n=10), large cell carcinoma in 4.3% (n=2), small cell carcinoma in 6.4% (n=3) and non small cell carcinoma in 17%(n=8). We found an OR of 1.09 (95% CI=0.35-3.42) and 0.19 (95% CI=0.03-1.02) for EPCA and ACA, respectively. However, these associations were not statistically significant.

Table 1. Demographic characteristics of the lung cancer patients and controls

Characteristics	Patients (n=47)	Controls (n=40)
Age, yrs (mean±SD)	56±10	39±11
Age, yrs, range	(30-75)	(20-65)
Male (n)	44	25
Female (n)	3	15
Smokers (n)	43	11
Quantity smoked (mean package/years)	38.2±3.2	19.5±4.3

Table 2. Frequency of GSTM1 genotypes in lung cancer patients and controls

	Patients N (%)	Control N (%)	OR (95%) CL	P value
GSTM1(+)	23 (48.9%)		17 (42.5%)	
GSTM1- null	24 (51.1%)	23 (57.5%)	1.30 (0.55-3.03)	0.55

Discussion

A number of studies have tried to establish links between polymorphic expression of different GSTs and lung cancer risk in different ethnic populations (10,15-18) and the results have been conflicting (15,19). One reason for the discrepancies could be the fact that most studies were conducted in different populations. However, none of the main characteristics of the subjects (i.e. race, histological type and level of smoking) explain satisfactorily the apparent discrepancies. Different histological subtypes of lung cancer, in particular may also be related to respective exposures or factors, and thus need to be analyzed separately (15,20).

The M1 variant of GST (GSTM1) detoxifies reactive intermediates of PAHs and other carcinogens. Although the relationship between GSTM 1 polymorphism and lung cancer has been studied by various investigators, the effect of GSTM null allele has not been explained clearly yet. A significant association of GSTM1 null genotype with lung cancer has already been observed in two large studies from Japan (21,22) and two from China (23,24). Furthermore, in a study in Caucasians, a significant association of lung adenocarcinoma with the GSTM1 null genotype was reported (25). In a meta-analysis study by Williams at al, it was shown that GSTM null allele was a risk factor for the development of lung cancer (26). A meta-analysis of 11 studies found an OR of 1.6 (95% CI= 1.26–2.04) for an association between the GSTM1 null genotype and lung cancer risk (27). In a meta-analysis study, it was reported that there was no statistically significant relationship between carrying GSTM null genotype and susceptibility to lung cancer but the number of patients carrying this genotype was greater in the lung cancer group (12). In our study, we found no statistically significant relation between GSTM null genotype and susceptibility to lung cancer. Additionally, the rate of GSTM null genotype was higher in the control group than in cancer patients.

GSTM1 locus is entirely absent in approximately 50% of Caucasians (28). GSTM1 null genotype has been shown in 31% to 66% of Asians, Indians and Caucasians (28-30). On the other hand, the frequency of GSTM1 deletion polymorphism for African-Americans was found to be 23% to 35% (31). This figure was 21% for Chileans (10). In the study by Öztürk and coworkers, GSTM1 null genotype incidence was found to be very similar in the control subjects and in patients with lung cancer (respectively 51.7% and 51.5%) in the Turkish population (28). In two other studies a prevalence of 34% and 18% for null polymorphism was reported in the Turkish population. The reason for the difference between these two studies was attributed to regional variation (32,33). In our study, the rate of GSTM1 null genotype was detected as 57.5%.

Our observations showed that carrying the GSTM1 genotype is not a risk factor alone for lung cancer. Large scale multicenter studies are necessary to obtain more reliable and correct results.

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