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The relationship between the risk of lung cancer and the exon 5 *(ILE105VAL)* polymorphism of glutathione S-transferase P1 (GSTP1) gene

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ABSTRACT

Lung cancer is one of the cancers which are seen all around the world and whose mortality rate is high. Many environmental factors and genotypes of individuals play an important role in the process of lung cancer. Previous epidemiologic studies have suggested that cancer risks are modified by some genetic polymorphisms. Glutathione S-transferase P1 (GSTP1) gene polymorphism is the most explored in all of these polymorphisms. GSTP1, the most abundant GST isoform in the lung, metabolizes numerous carcinogenic compounds including polycyclic aromatic hydrocarbons (PAH), a tobacco carcinogen. Utilizing a hospital-based case-control study, we investigated the association between GSTP1 polymorphisms at exon 5 with lung cancer risk in the Turkish population living in Mersin. The study population consisted of 50 lung cancer cases that were diagnosed at the Clinical Oncology Department of the Mersin University Hospital and 50 healthy controls matched for sex and age. Genomic DNA from patients and healthy controls was extracted from peripheral blood leukocytes and PCR-RFLP assay were used to genotype the GSTP1 polymorphisms. A statistically significant difference was observed only in the frequencies of the GSTP1 exon 5 allele and genotype between the control group and the case group (p=0.037). There was an association between the exon 5 mutant genotype (GG) and overall lung cancer risk (OR 7.55, 95% CI 1.127-50.596). [Turk J Cancer 2006;36(1):5-10].

KEY WORDS:

Lung cancer, genetic polymorphisms, GSTP1 gene

INTRODUCTION

Lung cancer is the most widespread and the most fatal type of cancer worldwide. Polymorphisms of glutathione S-transferase enzymes show differences according to the races in the world. The frequency of lung cancer in men is higher than women (1). The survival is rather low, only 15% of the individuals with lung cancer can manage to survive for 5 years. According to the WHO guidelines, lung cancer is histologically divided into 5 subtypes; squamous cell carcinoma, adenocarcinoma, small cell carcinoma, non-small cell carcinoma and adenosquamous carcinoma (2). Lung cancer generally appears after the age of 45 and its incidence increases by age. Together with smoking which is the most important risk factor (92% in men, 78% in women) conditions of labour, nourishment, age, gender, socioeconomic condition and genetic susceptibility have a big role on the etiology of lung cancer (3-5). Genetic factors consist of the interactions of tumor suppressor genes, oncogenes, the genes coding the enzymes of xenobiotic

metabolism and gene amplification (6). In the etiology of lung cancer, the role of genetic susceptibility is important (7). The incidence of lung cancer and polymorphisms of glutathione S-transferase enzymes show differences according to race.

Among the constituents of tobacco smoke, the polycyclic aromatic hydrocarbons (PAHs), such as benzo (a) pyrene, play a major role in the chemical lung carcinogenesis (8). Glutathione S-transferases (GSTs) consist of a super family of phase II metabolic enzymes, such as glutathione S-transferase P1 (GSTP1), that catalyze the conjugation of reduced glutathione (GSH- L-Ɣ-glutamyl-L-cysteinyl-glycine) with electrophilic groups of a wide variety of compounds including PAH, tobacco carcinogen (9). The human cytosolic GST isoenzymes are comprised of the four gene families and are classified according to their biochemical characteristics: alpha (GSTA), mu (GSTM), theta (GSTT) and pi (GSTP). A new form of GSTs named zeta (GSTZ1) has been discovered (10). Also, although chromosomal localization has not been determined exactly yet, there are studies on a mitochondrial glutathione S-transferase named GST kappa. GSTM1, GSTM3, GSTT2 and GSTP1 are polymorphic in the human populations. GSTP1 has been mapped to chromosome 11q13 and contains seven exons (11). The GSTP1 gene has a polymorphism at exon 5 (ile105val). This GSTP1 polymorphism is connected with an A→G substitution in the nucleotides of 313 causing amino acid change of the GST pi protein. It has been suggested that this genetic polymorphism of GSTP1 exon 5 has functional effects on the GST gene product resulting in reduced enzyme activity (12). GSTP1 is widely expressed in different human tissues and is the most abundant GST isoform in the lung. Thus, alterations in the structure, function or expression levels of GSTP1 due to genetic polymorphisms could alter the ability to detoxify carcinogens and modulate lung cancer risk. In the epidemiological studies about this matter, controversial results are reported. For instance; though there are studies reporting that the activity of GSTP1 gene in the individuals having valine variant is rather low against diol epoxides and especially benzo(a)pyrene diol epoxide (BPDE), in a contrary study it has been shown that GSTP1/val1054 has higher catalytic function against the carcinogenic epoxides than GSTP1/ile105 (8, 13-15).

A lot of studies have been carried out in order to determine the relationship between lung cancer and the polymorphism of exon 5 in GSTP1 gene. Some studies suggested that exon 5 polymorphisms were associated with lung cancer development (8, 15-22). On the other hand, other studies reported that polymorphisms at exon 5 were not related with lung cancer (23-29). These contradictory results necessitate more studies about this issue. The purpose of this study was to examine the association of the GSTP1 polymorphisms of exon 5 with lung cancer.

MATERIALS AND METHODS

The study population consisted of 50 lung cancer cases (43 male, 7 female; mean age 54.4±8.6) who were diagnosed at the Oncology Clinic of the Mersin University Hospital and 50 healthy controls (43 male, 7 female; mean age 51.8±8.5) matched for ethnicity, sex and age (±5 years). All individuals gave informed consent before participating in the research. All the study subjects were interviewed with a standard questionnaire for their demographic characteristics, previous family history of cancer, cigarette consumption and smoking habits. A venous blood sample was drawn from each individual. The samples were collected into tubes containing ethylenediaminetetraacetic acid (EDTA). DNA was extracted from whole blood by a salting out procedure (30).

Polymerase Chain Reaction - Restriction Fragment Length Polymorphisms (PCR-RFLP) assay was used to genotype GSTP1 exon 5 polymorphism. The specific primers for the GSTP1 exon 5 polymorphism were F 5'-GTA GTT TGC CCA AGG TCA AG-3' and R 5'- AGC CAC CTG AGG GGT AAG-3'. Amplification was performed in an automated thermal cycler (Techne Progene, Cambridge, UK). The PCR conditions were 5 min for initial denaturation at 94° C, 30 cycles at 94° C for 1 min for denaturation, 90 sec at 59° C for annealing and 90 sec at 72° C for extension, followed by 7 min at 72° C for final extension. The amplified product of 431 bp was digested with restriction endonuclease Alw26I (MBI Fermentas)

for 2 hours at 37° C. The gel visualizing system was used (Vilber Lourmat, France). The fragments were separated on a 3% agarose gel stained with ethidium bromide. The wild type (AA), heterozygous genotype (AG) and mutant genotype (GG) yielded 2 bands (329 and 107 bp), 3 bands (329, 222 and 107 bp) and 2 bands (222 and 107 bp), respectively (Figure 1).

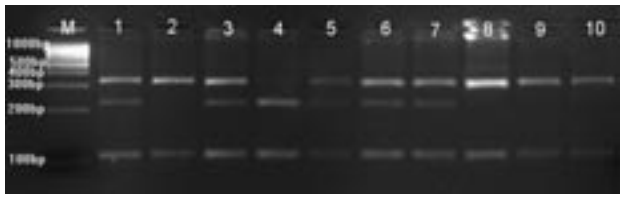

Fig 1. PCR-RFLP patterns of the exon 5 polymorphism. In the wild type sequence (AA), bands of 329 bp and 107 bp (lane 4) were generated, whereas in the homozygous mutant (GG), bands at 222 and 107 bp (lanes 2, 8-10) were produced. In the heterozygous AG, all three bands were present (lanes 1, 3, 5-7)

Statistical Analysis

All statistical analyses were performed using SPSS for Windows software. Allele and genotype frequencies were compared in lung cancer cases and healthy controls by using $\chi 2$ test. The Student's t test was used to compare quantitative demographic data in study objects. The strength of association was tested by multivariate logistic regression analysis. A p value of <0.05 was regarded as significant.

RESULTS

When we studied the relationship between age and the risk of lung cancer, the risk of lung cancer was found to increase with age (p=0.001). The average age of those with lung cancer is 54.4±8.6. Lung cancer risk increases 1.102 times with every 1 year increase above average. As for the relationship between smoking and lung cancer; 15 controls were non-smokers, 10 of were smoking one packet a day, and 25 were smoking more than one packet a day. On the other hand, 8 of those with lung cancer were nonsmokers, 14 of them were smoking one packet a day and 28 were smoking more than one packet a day. There was a relationship between smoking and the risk of lung cancer. This difference was more apparent for those who smoke more than a packet a day (p=0.001). As for the relationship between gender and lung cancer, 7 of our patients with lung cancer were female and 43 were male. The lung cancer risk was higher in men (p<0.001).

When the relationship between tumor types and genotype rates of exon 5 polymorphisms was studied, it was found that AA genotype was higher in squamous cell carcinoma and small cell carcinoma than adenocarcinoma. The AG genotype was found to be lowest in squamous cell carcinoma (p=0.035). The GG genotype was determined to be at similar rates in all tumor types. In this study, the proportion of women with adenocarcinoma and men with squamous cell carcinoma were found to be high in the total population of patients.

The allele and genotype distribution of GSTP1 exon 5 polymorphism is shown in table 1. There was a connection between lung cancer and exon 5 gene polymorphism genotype (p=0.047). Allele A of exon 5 gene polymorphism was found to be higher in the control group whereas allele G was found to be higher in the group with lung cancer

Table 1
Distribution of GSTP1 exon 5 genotypes in cases and controls

GSTP1 Genotypes	Controls N=50 (%)	Cases N=50 (%)	P Value
GSTP1 exon 5			
AA	33 (66)	19 (38)	
AG	14 (28)	24 (48)	
GG	3 (6)	7 (14)	0.037
A Allele frequency	80	62	
G Allele frequency	20	38	0.005

(p=0.005). Lung cancer risk was 7.55 times higher in homozygote (GG) individuals than those with the AA genotype (p=0.037).

DISCUSSION

In this study that investigated the role of polymorphic variants of glutathione S-transferase P1 (GSTP1) gene, it was found that there is a relationship between lung cancer and the exon 5 gene polymorphism genotype (p=0.037). Of exon 5 gene polymorphism frequencies, the frequency of allele A was found to be higher in the control group and allele G was found to be higher in the patients with lung cancer. Lung cancer risk was found to be 7.6 times higher in homozygote (GG) individuals than in those with AA genotype. When groups were evaluated in terms of exon 6 polymorphisms of the GSTP1 gene, no difference was observed between the groups.

Studies on tumor types reveal that the most common lung tumor is adenocarcinoma in women and squamous cell carcinoma in men (31,32). In this study also, the rates of women with adenocarcinoma and men with squamous cell carcinoma were found to be high in the total population of patients. There are a lot of studies about GSTP1 exon 5 polymorphism in different populations. Nazar-Steward et al. (13), in their study on patients with lung cancer, found a connection between lung cancer risk and 105 val allele (G) and especially homozygote genotype (GG). Ryberg (15) found that patients with lung cancer had a higher homozygote allele G frequency compared to the control group. In this study which measured DNA adduct levels as well, they also found that adduct level in individuals with allele G (val) was higher compared to individuals with allele A (ile). In addition, they also determined that lung cancer risk was two-fold in homozygote individuals for allele G. To-Figueras et al. (19) determined no important difference between the patient and control groups in terms of allele frequencies in exon 5 polymorphism. Lin et al. (20) reported that lung cancer risk was high in those with squamous cell carcinoma who had mutant valine allele (G) in exon 5 of the gene GSTP1. Stucker et al. (22), in their study on 251 patients with lung cancer, determined a two-fold lung cancer risk increase in individuals with homozygote 105val (GG) genotype. Katoh et al. (23) studied this A→G polymorphism on the 313th nucleotide of GSTP1 in terms of its connection with various cancer types in the Japanese population but could not find any relationship with lung cancer. To-Figueras et al. (26), in their study on 164 North-West Mediterranean Caucasian patients with lung cancer, determined no difference between the patient and control groups in terms of allele frequencies in exon 5 polymorphism. Lewis et al. (33), in their study analyzing the relationship between lung cancer risk and GSTM1, GSTT1 and GSTP1 polymorphisms, determined that there wasn't any connection between lung cancer risk and P1's exon 5 genotypes. Kihara et al. (34) reported that exon 5 polymorphism of the gene GSTP1 did not change lung cancer risk by itself but the interaction of GSTP1 exon 5 genotypes with GSTM1 null genotype increased the risk.

Findings in some studies indicate that these polymorphisms on the gene GSTP1 do not affect the risk of lung cancer by themselves individually, but they can change the risk only if seen together with other genetic polymorphisms. Miller et al. (16) reported that in case of the combination of GSTP1 GG (105ile) + GSTM1 null or GSTP1 GG (105ile) + p53 Arg/Pro, Pro/Pro genotypes, this variant of GSTP1 influences the increase in lung cancer risk. Jourenkova et al. (27) determined that GSTP1 105 val allele has no connection with the increase in lung cancer risk by itself, however in combination with GSTM1 null and GSTM3 AA polymorphisms it contributes to increased risk.

The fact that the greatest factor in lung cancer is smoking shows the importance of various enzymes that play a role in the excretion through xenobiotic metabolism of toxic materials, such as PAH contained in the cigarette smoke. GSTP enzyme is an important one that catalyzes phase II reactions of this mechanism. The importance of GSTP enzyme in lung cancer, in addition to being the most expressed enzyme to the lungs, is being the most important enzyme that metabolizes activated products of benzo(a)pyren, a carcinogenic material contained in the cigarette smoke, such as BPDE. As a result of single nucleotide changes in this polymorphism in the GSTP1 gene, isoleucine aminoacid is synthesized at the 105th codon of GSTP1 gene's exon 5 instead of alanine and this aminoacid change causes differences in the substrate binding active region of the enzyme. There are contradictions in most of the studies about the

way this difference affects enzyme activity. Watson et al. (35) reported that individuals with the variant allele of exon 5 polymorphism have significantly lower GSTP enzyme activity. Sundberg et al. (8) argue that valine aminoacid synthesis in related regions in GSTP polypeptide due to the changes in GSTP1 gene's exon 5 increases enzyme activity against BPDE, which is one of the major substrates of the enzyme and related to lung cancer because of its existence in the cigarette smoke, and thus aminoacid changes do not affect the risk for lung cancer. Contrary to this, Harries et al. (14) and Ryberg et al. (15) argue that valine variant has a low activity against polycyclic aromatic hydrocarbon diol epoxides, particularly against BPDE, and for this reason cancer risk will be higher in individuals with valine variant as detoxification potential will be lower. They have supported this argument with the finding that the frequency of valine variant is higher in patients with lung cancer compared to controls.

Our findings regarding the relationship between lung cancer and smoking, age and gender are consistent with the literature. It was determined in our study that GSTP1 exon 5 (ile105val) polymorphism is associated with the increase in lung cancer risk and the risk for lung cancer is about 7.5 times higher in individuals who have homozygote allele G. As a result of our evaluations taking into account the age, smoking habit, and tumor types of individuals; it is evident that the risk for lung cancer especially for adenocarcinoma is high in individuals who carry the mutant allele G of exon 5 of GSTP1 gene as homozygote. While adenocarcinoma is more prevalent in women, squamous cell carcinoma is more prevalent in men. The findings of our study parallels those of a major portion of literature about exon 5 polymorphism and its association with lung cancer. However, there is some literature arguing otherwise, stressing the effect of race in the relationship between these two polymorphisms and lung cancer. The findings regarding the effects of the GSTP1 polymorphisms on enzyme function and their relationship with lung cancer risk are contradicting. Therefore, more comprehensive studies evaluating the interaction of this polymorphism with genes related to lung cancer might prove useful in the future.

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