RESEARCH ARTICLE

Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) Polymorphisms and Lung Cancer Risk among a Select Group of Iranian People

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Abstract

Objective(s): Lung cancer, caused primarily by smoking, is one of the leading determinants of mortality throughout the world. Here we investigated the effects of polymorphisms in two enzymes, i.e., GSTT1 and GSTM1, related to the antioxidant defense line against carcinogens associated with lung cancer among a select group of Iranian people. **Materials and Methods:** One hundred and twenty lung cancer patients from two referral centers in Tehran, Iran, were recruited for comparison with 120 healthy controls. Genomic DNA was extracted from the FFPE tumor tissues of the select cases and peripheral blood buffy coats of healthy controls. The polymorphisms of GSTT1 and GSTM1 were investigated by multiplex polymerase chain reaction. **Results:** With the 240 samples studied, no specific relationship with lung cancer was discerned for the GSTM1 (P=0.35; OR=1/33; 95% CI=0.79-2.25) polymorphism, but the GSTT1 (P=0.005; OR=2.4; CI=1.32-4.35) gene polymorphism revealed a notable association on logistic regression, taking into account age and sex factors. Furthermore, the GSTT1 genotype distribution in patients with LSCC was different from that of healthy cases (P=0.006; OR=3.11; CI=1.38-7.04). The risk of developing lung cancer with the T0M1 genotype was 3.46 times higher than with T1M1 genotype (P=0.002; OR=3.46; CI=1.61-7.46). Moreover, the risk of developing LSCC cancer in people with T0M1 genotypes was significantly elevated (P=0.004; OR=4.5; CI=1.62-12.52). **Conclusion:** Unlike GSTM1, the GSTT1 genotype distribution is associated with the incidence of lung cancer in Iranian people. Different types of lung cancer appear to show various correlations with GST polymorphisms in this regard.

Keywords: Glutathione S-transferases T1 (GSTT1), Glutathione S-transferases M1 (GSTM1), Lung Cancer,

Introduction

World cancer statistics have shown that an estimated 42 million people in the world are affected by the disease, which is approximately over twice the rate in 1990 (Ritchie, 2018). The incidence of cancer in developing countries has had a significant impact on developed countries, accounting for 70% of deaths in these countries. Meanwhile, lung cancer has had the highest mortality rate in the world (Organization, 2018). Tobacco smoke is the leading cause of lung cancer (Islami et al., 2015). More than 30% of the world's population is exposed to the type of cancer caused by tobacco smoke (UK, 2012). Despite the mortality rate amongst non-smokers, various studies have indicated that other causes of mutation can also contribute to lung cancer. Furthermore, other factors including exposure to indirect tobacco smoke, environmental pollution from the workplace, heavy metals, exposure to industrial pollutants such as asbestos and radon, air pollution caused by fossil fuels such as Polycyclic Aromatic Hydrocarbons (PAHs), and the use of other compounds associated with this type of cancer, such as consumption of alcoholic compounds are also influential (Samet et al., 2009; Clément-Duchêne et al., 2010; García-Lavandeira et al., 2016).

All in all, all these factors are associated with lung cancer through environmental and genetic effects. The simultaneity of cigarette smoking and consumption of alcoholic beverages can have exacerbating effects on a variety of gene mutations associated with lung cancer because these two behaviors increase free radicals, and at the same time solubilize carcinogenic compounds

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(Bagnardi et al., 2010; García-Lavandeira et al., 2016). Biological contamination caused by PAH and its related compounds has also been well documented in the development of lung cancer, especially in CYPs and GSTs polymorphism (Fontana et al., 2009; Jiang et al., 2014; Peddireddy et al., 2016). As a lipophilic compound that can pass through the cell membrane , PAH damages the DNA as it converts to various carcinogenic compounds (Shen, 2016).

As a respiratory organ, lung is always exposed to peripheral oxygen and other sources of contamination in the surrounding environment. In order to counteract these carcinogens, antioxidant defense systems are organized into two phases. In the first phase, the Cytochrome P450 (CYPs) enzymes function collectively not only to activate but also to change the structure of compounds such as xenobiotics via reducing, oxidation and hydrolyzing them. Subsequently, it disables these compounds in the second phase via conjugating them with glutathione through Glutathione S-Transferases (GSTs) (Hukkanen et al., 2002; Jiang et al., 2014; Mota et al., 2015). GSTs preserve the stability of the cell's structure and prevent tissue damage, in this phase, through restricting the compounds that cause oxidative stress. Due to their sensitive function in counteracting oxidative stress, GSTs are highly exposed to polymorphism damage in their component substructures (Hayes et al., 2005; Tsuchida and Yamada, 2014).

The genotypes of GSTT1, GSTP1, and GSTM1 enzymes have been widely studied in recent years to evaluate individuals' susceptibility to common environmental conditions that cause cancer (Hezova et al., 2012; Uddin et al., 2014; Yu et al., 2016). GSTM1 and GSTP1 enzymes specifically detoxify active metabolites of PAH, predominantly Benzo[a]pyrene (BaP) and nitrogen-containing compounds (such as nitrosamines and aromatic amines), while GSTT1 enzyme is known to be involved in the metabolism of small compounds found in tobacco smoke including monohalomethanes and ethylene oxide (Hecht, 1999; Garte et al., 2007).

We examined, in the same article, the effects of GSTT1 and GSTM1 polymorphisms on glaucoma as a disease caused by oxidative stress (Kazemi Safa et al., 2014; Safa et al., 2015). Based on the increased prevalence of tobacco smoking (WHO, 2000-2025; Islami et al., 2015), and incidence of lung cancer in Iran (Almasi et al., 2016; Khazaei et al., 2017), we investigated, in this study, the distribution of polymorphisms in the GSTT1, and GSTM1 genes singly, as well as in combination, in lung cancer patients and healthy controls. Various types of lung cancer in a referral sample population were considered to determine whether any of the polymorphism confers an increased risk of developing lung cancer or not.

Materials and Methods

Patients and Tissue Specimens

The biological materials were provided by Iran National Tumor Bank and Masih Daneshvari Hospital. One hundred and twenty formalin fixed paraffin-embedded (FFPE) tissue blocks of lung cancer were used as study cases. Patients were diagnosed with lung cancer based

on a histopathological examination by at least two expert lung pathologists (Table 2). Histological types of lung cancer included non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LSCC), and lung adenocarcinoma (LAC). The other types of lung cancer, including large cell carcinoma (LCC), mesothelioma, and bronchial carcinoid, were addressed as other types in Table 3.

Demographic and clinical data were derived from medical records. None of the patients had received Neoadjuvant therapy. Subjects with chronic or acute inflammatory diseases in past six months or any other malignancies were excluded. For healthy controls, one hundred and twenty peripheral blood samples from individuals without any chronic or acute inflammatory diseases in past six months were recruited. The involvement of all healthy controls and lung cancer cases in the study began after their verbal consent or filling the required written consent forms. This study that was conducted with the approval of Lorestan University of Medical Sciences ethics committee, utilized protocols approved by the respective institutional review boards (ethic study number (Lums.rec.1394.1). Lung biopsies that were recognized for non-lung cancer by histopathologic examination with age and sex matched to patients group were selected as control group. Subsequently, both healthy and patient control groups were evaluated with regard to GSTT1, and GSTM1 gene polymorphisms.

DNA Extraction and PCR Methodology

DNA extraction of FFPE blocks of lung cancer tissue samples was done according to GeneAll® protocol kit, while the DNA extraction of the control group was performed based on the DNA extraction kit Cinna Gene protocol from extracted WBC taken from buffy coat of peripheral blood samples. To identify the presence of GSTT1 and GSTM1 genes, we used a multiplex polymerase chain reaction (PCR) on the Dihydrofolate Reductase (DHFR) gene as an internal control to detect the null genotype of GSTT1 and GSTM1 genes.

Primer sequences were designed using the AlleleID7 software (Premier Biosoft Corporation, USA), and then synthesized by TakapouZist Company (Iran). Primers have been indicated in Table 1. PCR reaction was carried out in an ultimate volume of 25 µl containing 12.5 µl of ready-made Master mix (Thermo Fisher Scientific) from each of the primers of forward, and reverse 1.5 mmol/L and 7.5 mmol/L of H_2O and 2 mmol/L of DNA at a concentration of 50 -200 ng was added to the material.

Amplification was performed through preliminary denaturation at 94 ºC for 5 minutes, followed by f 35 PCR cycles at 94 ºC for 30 seconds, 64 ºC for 45 seconds and 72 ºC for 1 minutes, and an ultimate extension of 72 ºC for 5 minutes. Amplified products were identified by electrophoresis in a 2% agarose gel, and were stained with 0.5 µg/ml ethidium bromide. Product size was 215 bp, 480 bp, and 280 bp for GSTM1, GSTT1, and DHFR respectively (Figure 1).

T1M1 genotype was specified by two bands of 480 bp for GSTT1 and 215 bp for GSTM1, the T1M0 genotype showed one band of 480 bp, while it was

215 bp for T0M1 genotype. For the T0M0 genotype (deletion of two genotypes), no bands were indicated, hence the use of DHFR as internal positive control was essential to diagnose the null genotype from aborted PCR responses.

Statistical Analysis

Data analyses were performed using SPSS Statistics software (version 16; SPSS Inc., Chicago). The Chi-square test was carried out to compare categorical variable and independent t-test to continue with a significant difference between the two groups (P<0.05). Lung cancer risk was estimated as odds ratios (OR) and 95% confidence interval (95% CI) using logistic regression analysis.

Results

A total of 240 study subjects comprising 120 lung cancer patients and 120 healthy controls were studied. Demographic information of all participants is indicated in Table 2. Based on the study results, there was no significant difference concerning the mean age (P=0.13) between the two study groups, though they were not sexually identical (P=0.019) (Table 2).

The statistical results demonstrated that the probability of encountering the GSTM1 null genotypes in lung cancer patients was 33% higher than healthy individuals to this genotype, yet it was not statistically significant (P<0.05). Utilization of logistic regression, adjusting for age and sex, indicated that there was no significant relationship between the incidence of lung cancer and GSTM1 polymorphism (P=0.35; OR=1/33; 95% CI=0.79-2.25). Moreover, the GSTT1 genotype distribution in lung cancer was 2.4 times higher than healthy individuals (P=0.005; OR=2.4; CI=1.32-4.35). Furthermore, the relationship between genotypes and
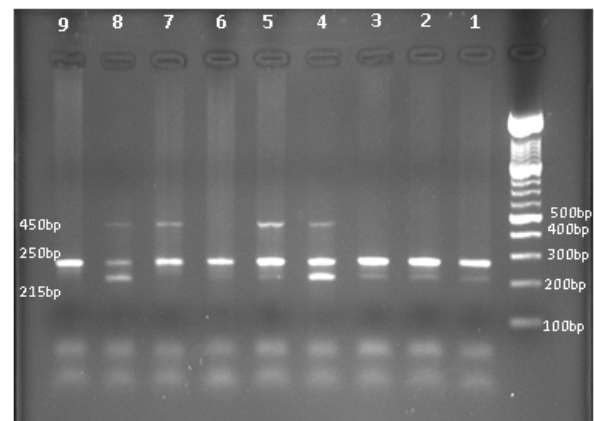

Figure 1. Amplified PCR Products of GSTT1, GSTM1 and DHFR Gene Polymorphism. Lines (4, 5 and 8) heterozygous for GSTT1 and GSTM1. Lines (7) were homozygous deletion for GSTT1. Lines (1, 2, 3 and 7) were homozygous deletion for GSTM1, and lines (6 and 9) were deletion for both GSTM1 and GSTT1.

different types of lung cancer has been studied (Table 3). This analysis indicates that GSTT1 genotype distribution in patients with LSCC of lung is 3.11 times higher than healthy individuals, which is statistically significant (P=0.006; OR=3.11; CI=1.38-7.04), considering the fact that GSTT1 genotypes distribution in patients with other types of lung cancers is 2.7 times higher than healthy individuals, which is also statistically significant (P=0.035; OR=2.7; CI=1.07-6.8). There was no correlation between the distribution of these genotypes and NSCLC/LAC (P>0.05) (Table 3).

Finally, multivariate logistic regression analysis, adjusting for age and sex, revealed that the risk of developing lung cancer in T0M1 genotypes was 3.46 times higher than that of T1M1 genotype (P=0.002; OR=3.46; CI=1.61-7.46), and the risk of developing other types of

Table 1. Primer Sequences

Primers Sequence		Annealing	Reference
GSTM1 forward	5'– GAA CTC CCT GAA AAGCTA AAGC-3'	62 C	
GSTM1 reverse	5'- GTT GGG CTC AAA TAT ACGGTG G-3'		
GSTT1 forward	5'– TTC CTT ACTGGT CCT CAC ATC TC-3'	61C	(Abdel-Rahman et al., 1996)
GSTT1 reverse	5'– TCA CCGGACAT GGC CAG CA-3'		
DHFR forward	5'-GGA ATG GAG AAC CAG GTC TT-3'	62C	
DHFR reverse	5'-GCA TGT CTT TGG GAT GTG GA-3'		

Table 2. Demographic Information of the Study Papulation

Study group	Control group (n=120)	Lung cancer group (n=120)	P-value
Sex			
Men, n (%)	90 (75.0%)	72 (60.0%)	0.019
Women, n (%)	30 (25.0%)	48 (40.0%)	
Lung cancer types			
Non-Small Cell Carcinoma		19 (15%)	
Adeno Carcinoma		39 (32%)	
Squamous Cell Carcinoma		36 (30%)	
Other Types		26 (21%)	
Age (years), Mean ± SD	55.2167 ± 12.63660	54.4583 ± 9.98730	0.13

Table 3. Genotype Distribution and Risk Lung Cancer Associated with GSTM1 and GSTT1 Polymorphism

Study group	n	GSTM1				GSTT1			
		Present, n (%)	Null, n (%)	OR (95% CI)	P-value	Present, n (%)	Null, n (%)	OR	P-value
Control	120	79 (65.6)	41 (34.2)	Reference		98 (81.7)	22 (18.3)	Reference	
Case	120	71 (59.2)	49 (40.8)	1.33 (0.79-2.25)	0.35	78 (65)	42 (35)	2.4 (1.32-4.35)	0.005
NSCC	19	11 (57.9)	8 (42.1)	1.52 (0.54-4.24)	0.42	14 (73.7)	5 (26.3)	1.32 (0.4-4.26)	0.638
LAC	39	23 (59.9)	16 (41)	1.4 (0.66-3)	0.38	27 (69.2)	12 (30.8)	1.78 (0.71-4.13)	0.174
LSCC	36	23 (63.9)	13 (36.1)	1.1 (0.49-2.46)	0.81	21 (59.3)	15 (41.7)	3.11 (1.38-7.04)	0.006
Other Types	26	14 (53.8)	12 (46.2)	1.7 (0.7-4.14)	0.25	16 (61.5)	10 (38.5)	2.7 (1.07-6.8)	0.035

P < 0.05 was statistically significant, OR odds ratio and (95% CI) confidence interval were calculated by logistic regression and adjusted for age, gender

lung cancer in people with T0M0 genotypes was 2.67 times higher than those with T1M1 genotypes (P=0.048; OR=2.67; CI=1.01-7.07). The risk of developing LSCC cancer in people with T0M1 genotypes was 4.5 times higher than those with T1M1 genotypes, which was statistically significant (P=0.004; OR=4.5; CI=1.62-12.52). Moreover, the risk of developing other types of lung cancer with T0M1 genotypes was 3.62 times higher than those with T1M1 genotypes, which was also was statistically significant (P=0.04; OR=3.62; CI=1.06 -8.07). On the other hand, the risk of developing other types of lung cancer in people with T0M0 genotypes was 4.37 times higher than those with T1M1 genotypes that were statistically significant (P=0.042; OR=4.37; CI=1.6-18.08). In other cases, correlation between different genotypes and risk of other types of lung cancer were not identified. (P> 0.05) (Table 4).

Discussion

Several studies have investigated the relationship between polymorphisms of glutathione S-transferase and lung cancer. In this study, however, the notable association between the genetic deletion of GSTM1 genotype and risk of lung cancer was not perceived, while GSTM1 null genotype was 33% higher than healthy individuals. Meanwhile, the genetic deletion of GSTT1 and the T1M1 combination genotypes were perceived to be lung cancer risk factors, more especially in LSCC.

Various studies have indicated relationship between GSTT1 null deletion and the risk of lung cancer (Sorensen et al., 2004; Yang et al., 2014; Wang et al., 2015; Zhao et al., 2015). Contrary to the results obtained in this research, the relation between GSTT1 gene polymorphism and lung cancer was not confirmed in other studies (Abbas et al., 2004; Sharma et al., 2015). Aa Sharma et al., (2015) had already confirmed in their investigation on a select number of subjects among Indian population, Jiang et al., (2014) suggested that there is an essential alteration in genotypic frequency of GSTT1 of healthy Mongolian and Han subjects in the population of Inner Mongolia Region.

Furthermore, in the present study, GSTT1 genotype that maintained the correlation with LSCC and other types of lung cancer, showed a significant increase of 3.11 and 2.7 fold respectively, wherein there was no correlation between these genotypes distribution and NSCLC/LAC.

After stratification into histological subtypes, there are some studies show a remarkable association between LAC/SCLC and GSTT1 null (P=0.002) and variant GSTP1 genotypes (Sharma et al., 2015), like another study that found a correlation between GSTT1 and NSCLC (P= 0.008) (Peddireddy et al., 2016). In the study by Zhang et al., (2014) in non-small cell lung cancer, the observed frequencies were (OR=2.071, P=0.009) for GSTM1 null genotype, (OR=1.900, P=0.024) for GSTT1 null genotype, and (OR=3.258, P=0.003) for GSTM1/T1 null genotype, which have been significantly greater in NSCLC group than in the control group. Jiang et al., (2014) suggested that there was a statistically surprisingly relationship between GSTM1 and lung cancer in Mongolians and Han population. Although no statistically significant difference was observed in relation to the types of lung cancer and the deletion of this polymorphism, an increase of approximately 1.5 fold was confirmed. In the study conducted on the North Indian population, a relationship between GSTM1 invalid genotype and lung malignancy (P=0.005) was observed, and this risk was greater in adenocarcinoma (LAC) group (Sobti et al., 2008; Sharma et al., 2015). In contrast, some studies have led to different results in SCLC patients (P= 0.83) (34), e.g., the research carried out by Peddireddy et al., (2016) it was confirmed that GSTM1 genotype could protect against the risk of lung cancer.

Based on the results obtained in this research and similar studies, GSTT1 genotype has always been referred to as being capable of having effective antioxidant activity, and gene deletion in the null state with its complete inactivation could influence its inactivity in the body. This is especially observable in different types of carcinogens including PAHs and similar compounds such as monohalomethane and ethylene oxide in tobacco smoke, one of the substrates of which is GSTT1 (Landi, 2000; Hayes et al., 2005).

In contrast with the results of previous studies (Yang et al., 2015; Zhunussova et al., 2015; Gao et al., 2017; Malik et al., 2017), in this study, we did not find a correlation between the GSTM1 gene polymorphism the risk of lung cancer in Iranian people, while the probability of encountering the GSTM1 null genotypes in lung cancer patients was 33% higher than healthy individuals with this genotype, although it was not statistically significant (P<0.05). The same result had already been obtained in

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Table 4. Genotype Distribution and Risk Lung Cancer Associated with Combination Genotype of T1M1 Polymorphism

Genotype	Control n (%)	LC n (%)	OR, CI 95%	NSCC, n (%)	OR, CI 95%	LAC, n (%)	OR, CI 95%	LSCC n (%)	OR, CI 95%	Other Types n (%)	OR, CI 95%
T1M1	66 (55)	41 (34.2)	Reference	6 (31.6)	Reference	15 (38.2)	Reference	12 (33.3)	Reference	8 (30.8)	Reference
T1M0	33 (27.5)	36 (30)	1.72 (0.93-3.2)	7 (36.8)	2.28 (0.68-7.59)	12 (30.8)	1.53 (0.63-3.67)	9 (25)	1.49 (0.57-3.9)	8 (30.8)	2.06 (0.7-6.03)
T0M1	13 (10.8)	30 (25)	*3.46 (1.61-7.46)	5 (26.3)	3 (0.75-11.99)	8 (20.5)	2.12 (0.72-6.26)	11 (30.6)	*4.5 (1.62-12.52)	6 (23.1)	*3.62 (1.06-12.4)
T0M0	8 (6.7)	13 (10.8)	*2.67 (1.01-7.07)	1 (5.3)	1.41 (0.14-14.01)	4 (10.3)	2.4 (0.62-9.2)	4 (11.1)	2.76 (0.72-10.65)	4 (15.4)	*4.37 (1.56-18.01)

P < 0.05 was statistically significant, OR odds ratio and (95% CI) confidence interval were calculated by logistic regression and adjusted for age, gender

some studies conducted on Chinese (Liu et al., 2015), and Turkish population (Demir et al., 2007). However, among East Asian populations, the GSTM1 null genotype seemed to be the foremost risk factor for lung cancer (Carlsten et al., 2008; Zhao et al., 2014). Liu et al., (2014)reported that GSTM1 null genotype was expressively associated with lung malignancy possibility in Asian population. An important correlation of GSTM1 null (P=0.035, I2=40.58%) with lung cancer was determined in a study carried out on Indian population by Dey et al., (2015). Moreover, in the meta-analysis studies by Malik et al., (2017) in various populations during the last ten years, the researchers found a correlation between GSTM1 (OR=1.31) and the risk of lung cancer. In the Chinese population, GSTM1 null genotype among smokers had a developed lung cancer risk than non-smokers (Liu et al., 2014a). In another study, they found an accelerated risk of GSTM1 null genotype for lung cancer in stratified analyses of smoking status and histological form. The rate of GSTM1 null genotype was 57.7% and 50.1% in lung cancer and control group respectively (Yang et al., 2015).

Furthermore, contrasting results were obtained in combined null genotypes of GSTT1 and GSTM1 in the study by Kui Liu et al., (2014a) GSTT1 (P<0.001) and GSTM1 null genotype among smokers and lung cancer patients significantly elevated (P<0.001). in the Chinese population. Gao et al., (2017) observed from the combined consequences of GSTM1 and GSTT1 polymorphisms that lung cancer odds was perceived in Caucasians (OR=1.23, 95% CI=1.07-1.41), Asians (OR=1.24, 95% CI=1.10-1.41) and Indians (OR=2.53, 95% CI=1.61-3.98), which were associated with expanded lung cancer hazard. Individuals with T0M0 genotypes had an increased risk for lung cancer (OR=2.0), but in the case of SQCC it was 1.6-fold (OR=1.6) (Sobti et al., 2008). In the Slovakian population, M0T1 genotype for lung cancer showed a risk which was twice higher (OR=1.98; P=0.006) (Matakova et al., 2009).

No correlation between deletion of GSTM1 and GSTT1 and the rise of lung cancer risk was confirmed by Masood et al., (2016) in Pakistan. Moreover, no correlation was found between M1/T1 (P=0.83) null and lung cancer risk in another study conducted on Brazilian population (Honma et al., 2008).

In the study conducted by Masood et al., (2016) no significant relationship was found between genetic deletion in GSTT1 and GSTM1. Similar studies have been carried out in Belarus, southern India, and Turkey (Sreeja et al., 2005; Chakova et al., 2009; Ada et al., 2012). In another study by Atinkaya et al., (2012) in the Turkish population, there was no statistical distinction amongst groups in relations of GSTT1 null genotype and GSTM1 null genotype. Atinkaya et al., (2012) indicated in their research conducted on Turkish population that there was no statistical distinction amongst groups concerning the relations between GSTT1 null genotype and GSTM1 null genotype (Uddin et al., 2014). There was no statistical difference in the main genotypes that had been detected for the carcinogen metabolism genes in relation to GSTM1 null genotype and GSTT1 null to the lung cancer odds in

Asturias (López-Cima et al., 2012).

This study suffers from several limitations such as neglecting habitual and environmental risk factors including tobacco smoking, air pollution, and occupational exposures beside genetic variation in glutathione S-transferases (GSTs) which have been investigated. Multicenter ongoing studies could provide more comprehensive results on the correlation between GSTs polymorphism and types of lung cancer. The comparison between Glutathione S-transferase gene expression and presence of GSTs such as sensitive biomarker of blood toxicity could practically demonstrate the effect of this polymorphism on reducing its antioxidant activity. Finally, the comparison between the gene polymorphisms in phases I and II antioxidant defense against carcinogens could be highly effective with regard to this disease due to substances in tobacco smoke and air pollution.

In conclusion, our results confirmed that individuals carrying both null genotypes for GSTM1 and GSTT1 genes had a greater risk of lung cancer than those with one of these genes in Iranian people. It was also observed that GSTM1 null genotype did not have a significant correlation with lung cancer in various studies. Moreover, GSTs polymorphism has indicated different correlations with each type of lung cancer, and the risks of two GSTM1 and GSTT1 genetic polymorphisms as sensitive genotypes were considered as genetic modifications of the risk for lung cancer.

Acknowledgements

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