



Research Article

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Glutathione S-transferase Isoenzymes in Lung Carcinoma Patients receiving Platinum-based Chemotherapy and Effects of Ethyl Alcohol and Smoking on Treatment Response



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Abstract

Mostly observed in men, lung carcinoma, mostly observed in men, is one of the most frequent cancer types in Turkey and in the world. About two thirds of the lung carcinoma patients have inoperable lesions. 113 primary lung cancer patients who were receiving platinum based treatment at Chest Diseases and Chest Surgery Center of Ankara Atatürk Hospital were enrolled for the study. It was identified that 49 (43%) patients had *GSTM1* gene deletion out of a group of 113. It was identified that 42 (37%), 34 (30%) and 10 (9%) patients had *GSTP1* Ile₁₀₅/Ile₁₀₅ (wild type) genotype, Ile₁₀₅/Val₁₀₅ (heterozygote) genotype and Val₁₀₅/Val₁₀₅ (homozygote mutant) genotype, respectively out of a group of 113. *GSTP1* is the most strongly expressed isoenzyme in the human lung. In this study, the responses to cisplatin based chemotherapy of homozygous and heterozygous patients with *GSTP1* exon5 variant allele were observed to be similar to the responses of the patients carrying wild type genotype. It is displayed that wild type genes have positive effect on the response to treatment. This result yields that it is important to evaluate the effect of combination of GST genotype, alcohol ingestion amount, smoking frequency on the response to treatment

Keywords: Glutathione S-transferase; Lung Carcinoma; Platinum-based Chemotherapy

Introduction

Trachea, Bronchial, Lung
Prostate
Bladder
Colorectal
Stomach
Larynx
Non-Hodgkin Lenfoma
Kidney
Pancreatic
Brain, nervous system

MEN

Distribution of age-standardised incidence for the first frequently observed 10 cancers among men (United Data Base, 2009)(World's Standard population, among 100.000 persons)

Figure 1: 2009 Data from MoH Cancer Department.

Mostly observed in men, lung carcinoma, mostly observed in men, is one of the most frequent cancer types in Turkey and in the world, and it ranks the first in Turkey in terms of mortality from

cancer (Figures 1 & 2). About two thirds of the lung carcinoma patients have inoperable lesions. Even if these patients receive the most effective chemotherapy regimen, a significant part of

them dies within a year. "Non-small cell lung carcinoma" patients which constitute 80-85% of the carcinoma cases, respond to chemotherapy is 30-50% [1-3]. For this reason, it is very important

to conduct a search for the failure of chemotherapy in treatment of these patients.

Breast
Thyroid
Colorectal
Corpus of the uterus
Trachea, Bronchial, Lung
WOMEN
Stomach
Ovarian
Non-Hodgkin Lymphoma
Brain, nervous system
Pancreatic
Uterus cervix

Distribution of age-standardised incidence for the first frequently observed 10 cancers among women (United Data Base, 2009)(World's Standard population, among 100.000 persons)

Figure 2: 2009 Data from MoH Cancer Department.

Within this respect, the studies on mechanisms of drug resistance have been intensified recently and it is found that certain factors play roles in chemoresistance of cancer cells. It is thought that the glutathione s-transferases (GST) may be one of these factors. Catalyzing phase II reactions, GSTs are a superfamily of a dimeric nature and have a significant role in cellular defense system. GSTs protect cellular macromolecules from damages by catalyzing the conjugation of toxic and carcinogenic electrophilic molecules with glutathione [4]. Certain data indicate that GSTs determine the cytotoxic effect of various chemotherapy drugs. GSTs consist of 8 subfamilies namely as (alpha, [α]; kappa, [κ]; mu, [μ]; pi, [π]; sigma, [σ]; theta, [θ]; zeta, [ζ]; and omega, [ω]) and each class may have different variant isoforms. GSTP1 is the most strongly expressed isoenzyme in the human lung [5]. GSTM1 and GSTT1 isoenzymes are expressed in the lung, though lesser. GSTP1 is known as to be the most expressed GST in non-small cell lung tumors [6]. It is determined that GSTP1 levels of drug resistant tumors are high. Among these, non-small cell lung tumors are remarkable. It is argued that GSTP1 directly participates in the detoxification of platinum components and that it plays a significant role in both intrinsic and acquired resistance to platinum components [7]. However, there are studies demonstrating that platinum resistance is not associated with GSTP1. Similar contradictory results were presented for ovarian carcinoma.

One of the important reasons for not being able to demonstrate the correlation between GSTP1 expression and platinum components at any time, may be the polymorphism of *GSTP1* gene. It is well-known that *GSTP1* gene is polymorphic. Three different alleles were described; *GSTP1**A (wild type), *GSTP1**B and *GSTP1**C. The *GSTP1**B variant shows an A→G transition at position +313, codon 105, exon 5, changing the amino acid Ile to Val [8]. The *GSTP1**C allele has 2 transitions, the same as those

observed in the *GSTP1**B allele and a second one, located at position +314, codon 106, exon 6, changing the amino acid Ala to Val. The Ile₁₀₅→Val₁₀₅ substitution in the *B and *C variants reduces the catalytic activity of the enzyme [5] [9]. Thus, the carriers of mutant alleles can not metabolize carcinogens sufficiently, which could be responsible for a higher susceptibility to cancer.

Moreover, it is reported that GSTM1 and GSTT1 may be effective in the formation of several carcinomas, including lung carcinoma, and in treatment responses [10]. Since *GSTM1* and *GSTT1* genes are also polymorphic, lack of these genes results in loss of enzyme activities. Reduction in and loss of the activities of these polymorphic GSTs may decrease the detoxification of platinum components. This situation, by strengthening the cytotoxic effects of components and thus improving the treatment activities, increases the ratio of patient's response to the drugs.

Chronic alcohol ingestion disrupts mitochondrial structure and function, results in suppression of respiration, increasing of mitochondrial reactive oxygen species, decreasing of ATP and interruption of fatty acid metabolism. During alcohol metabolism, ethanol is converted into acetaldehyde by alcohol dehydrogenase (ADH), and acetaldehyde is changed into acetate in mitochondria by mitochondrial aldehyde dehydrogenase (ALDH). Both reactions use NAD⁺ as a co-factor. Later on, this is also converted into NADH. Alterations in cellular NAD⁺/NADH ratio caused by alcohol lead to decrease in ATP synthesis. As a result, disruptions in electron transport chain will lead to elevation of mitochondrial superoxide anions, H₂O₂ and hydroxyl radicals. Alcohol also has effects on cellular antioxidant defence mechanisms as well as on the formation of reactive oxygen species. It is demonstrated during the studies with rat model that chronic alcohol ingestion decreased the cellular GSH pool of alveolar Type 2 (ATII) cells and this resulted in the decrease in surfactant synthesis and

secretion which is vital [11]. The balance of intracellular oxidants and antioxidant systems is critical in the regulation of receptors and cytokines in the immune response. Decreased surfactant processing will lead to decreased barrier integrity and increased apoptosis. Alcohol induced reactive oxygen species generation and GSH oxidation may alter the response of other immune cells. Decrease in phagocytic capacity may be demonstrated as an example of this [12].

Effect of alcohol ingestion on systemic immune response was searched by using obese diabetic mice model and it was demonstrated that adaptive immune response was specifically suppressed in case of inflammation. It was demonstrated that low-dose alcohol ingestion disrupted immune response while it decreased the inflammatory response [13]. In another study carried out by using mice model demonstrated that short-term administration of alcohol negatively affected antigen presentation activity towards B cells [14].

Many studies were carried out concerning the role of smoking on the etiology of lung carcinoma. Various studies were conducted in order to search whether smoking or quitting smoking interferes with the response to treatment following the start of lung cancer treatment. It was observed that stopping smoking had very small positive effect on treatment while continuing to smoke decreased the effect of treatment and increased the recurrence frequency [15]. It is observed in mice model that nicotine decreased the chemotherapeutic effect of gemcitabine, resulted in tumor progression and elevated metastasis frequency although nicotine, itself, is not a carcinogen substance [16]. Human lung epithelium was used experimentally during the in vitro study and it was indicated that long term smoking elevated the resistance to chemotherapy in lung cancer. This study showed that decrease in Smad3 expression elevated carboplatine resistance in non-small cell lung cancer [17]. It is demonstrated through these studies that stopping smoking is mandatory for the success of lung cancer treatment. It was demonstrated that other factors also had role in prognosis of treatment during cisplatin based treatments. Polymorphisms in DNA repair genes and methylation of checkpoint genes in circulating serum DNA could have important predictive roles in survival in cisplatin-based treatment [3].

Platinum-based drugs such as cisplatin, carboplatin and oxaliplatin are frequently used in the treatment of cancer. Cisplatin is an organic, platinum derived drug which is different from other antineoplastics in terms of its structure. During the experiments conducted in order to examine the effect of electrical field which is formed in a liquid environment through platinum electrodes, on the growth of *e. coli*, it is incidentally noticed that platinum derivatives which were transferred into liquid from the electrodes had antibacterial and antineoplastic effects and thus cisplatin was discovered. Only its *g*is isomer is cytotoxic. It is not a period specific drug. It functions as an alkylating agent. It

binds to DNA with covalent bonds via N-7 of guanine and adenine. Cisplatin affects DNA in two ways as it binds with DNA to form intrastrand (90%) and interstrand (10%) crosslinks. This results in DNA adduct formation or DNA synthesis and transcription. Binding to nucleoproteins and cytoplasmic proteins, it may result in cytotoxic effects [18].

Carboplatin is a cisplatin analogue with an antineoplastic activity like cisplatin, but having less emetic effect and decreased renal toxicity, ototoxicity and neurotoxicity. Carboplatin shows effect as it is transformed into cisplatin after it is injected to the organism. Carboplatin binds with DNA to form intrastrand (more than 90%) and interstrand (less than 5%) crosslinks. It inhibits DNA synthesis and function through forming DNA adduct. Furthermore, it prevents the transcription. Binding to nuclear and cytoplasmic proteins, it results in cytotoxic effects. Glutathione and glutathione-related enzymes increase its inactivation.

Establishing whether these polymorphic genes affect the responses to platinum-based chemotherapy in lung carcinoma is important in terms of being able to plan the treatment forms that are effective on personal basis, as a result. Thus, the aim of this study is to conduct a research on the effects on response of *GSTM1*, *GSTP1* and *GSTT1* gene polymorphisms of both small cell and non-small cell lung carcinoma patients receiving platinum based treatment when these gene polymorphisms are associated with alcohol ingestion and smoking.

Materials and Methods

113 primary lung cancer patients who were receiving platinum based treatment at Chest Diseases and Chest Surgery Center of Ankara Atatürk Hospital were enrolled for the study. Certain diagnostic methods were used for staging small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC): direct chest graphy, lung, surrenal and brain computed tomography, bone scintigraphy were used as imaging methods and fine-needle aspiration biopsy, bronchoscopic biopsy and biopsy methods applied through surgical operation were used for pathological diagnosis. Whether the patients responded to chemotherapy was evaluated radiologically in accordance with the criteria of World Health Organisation (WHO). During the evaluation of the replies, patients who replied the questions fully and partly were accepted as the patients who replied the questions. Stable patients and patients with progression were included in the group of patients who did not reply.

The followings are the chemotherapy protocols used in lung cancer: 1) Gemcitabine+ Cisplatin 2) Docetaxel+ Cisplatin 3) Vinorelbine+ Cisplatin 4) Paclitaxel+ Carboplatin 5) Etoposide+ Cisplatin. During our study, the patients received treatment of at least two cures or more with 21 day-intervals. The radiological evaluations in accordance with WHO criteria were started to be conducted following the second cure.

WHO tumor response criteria:

Complete response: Disappearance of all symptoms radiologically.

Partial response: Tumor burden is decreased by $\geq 50\%$.

Stable disease: Present tumor burden increased $<25\%$ and decreased $<50\%$.

Progressive disease: Development of new lesions or growth of lesions $\geq 25\%$.

Ethyl alcohol ingestion amounts were defined according to the CDC criteria. Accordingly, accepting that one standard unit has 14 gr pure alcohol by volume, if the daily ingestion of alcohol is 2 units the male patient is considered as a medium drinker; the same applies for women whose daily ingestion of alcohol is 1

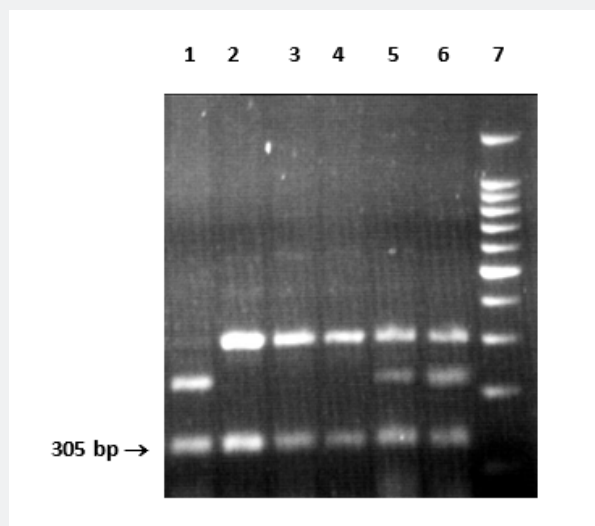
unit. Those whose daily ingestion is less than these amounts are accepted as light drinkers, and more than these amount, as heavy drinkers. Genomic DNA was isolated by (Promega) kit which is prepared according to the method of [19]. *GSTM1* and *GSTT1* genotypes were identified by multiplex PCR method as defined by [20]. Ile105Val and Ala114Val mutations in *GSTP1* gene were made through PCR and RFLP methods [21].

Statistical Analysis

Chi-square analysis was used in order to demonstrate whether the response to treatment was associated with smoking habit and alcohol ingestion of the person, with *GSTP1* exon5 and *GSTP1* exon6, *GSTT1* and *GSTM1*. Logistic regression analysis was used for determining the possible factors having effect on non-response. SPSS Statistics 20.0 was used during analysis.

Results

GSTM1 Genotype findings



1st column demonstrates *Val*₁₀₅/*Val*₁₀₅ genotype corresponded to two-bands of 138 and 222 bp, 2nd, 3rd, and 4th column demonstrates *Ile*₁₀₅/*Ile*₁₀₅ genotype corresponded to two bands of 138 and 305 bp, 5th and 6th column demonstrates *Ile*105/*Val*₁₀₅ genotype corresponded to three bands of 138, 222 and 305 bp, 7th column demonstrates DNA marker corresponded to 100 bp.

Figure 3: The result of electrophoresis which was used to determine the *GSTP1* *Ile*105Val genotype.

It was identified that 49 (43%) patients had *GSTM1* gene deletion out of a group of 113. The result of electrophoresis which was used to determine the *GSTM1* genotype is displayed in Figure 3.

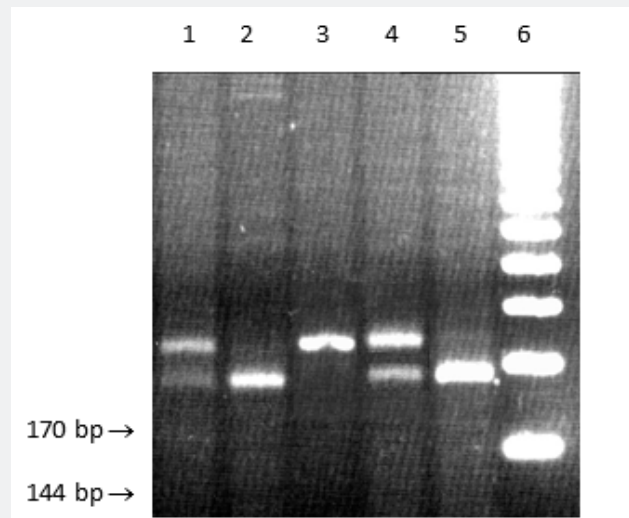
GSTP1 Ile105Val genotype findings:

It was identified that 42 (37%), 34 (30%) and 10 (9%) patients had *GSTP1* *Ile*₁₀₅/*Ile*₁₀₅ (wild type) genotype, *Ile*₁₀₅/*Val*₁₀₅ (heterozygote) genotype and *Val*₁₀₅/*Val*₁₀₅ (homozygote mutant) genotype, respectively out of a group of 113. The result of

electrophoresis which was used to determine the *GSTP1* *Ile*105Val genotype is displayed in Figure 3.

GSTP1 Ala114Val genotype findings:

It was identified that 64 (56%) and 11 (10%) patients had *GSTP1* *Ala*₁₁₄/*Ala*₁₁₄ (wild type) genotype and *Ala*₁₁₄/*Val*₁₁₄ (heterozygote) genotype, respectively out of a group of 113. 11(10%) patients were identified to have *Val*₁₁₄/*Val*₁₁₄ (homozygote mutant) genotype (Figure 4).



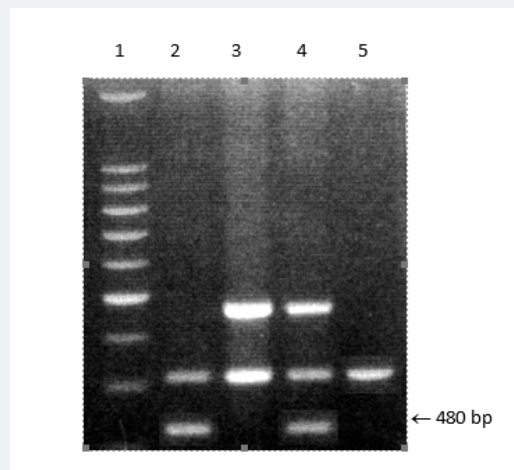
1st and 4th column demonstrate *Ala*₁₁₄/*Val*₁₁₄ genotype corresponded to two-bands of 144 and 170 bp, 2nd, and 5th column demonstrates *Ala*₁₁₄/*Ala*₁₁₄ genotype corresponded to a single band of 144 bp, 3rd column demonstrates *Val*₁₁₄/*Val*₁₁₄ genotype corresponded to a single band of 170 bp, (this band is taken from a different sample other than the study group) 6th column demonstrates DNA marker corresponded to 50 bp.

Figure 4: The result of electrophoresis which was used to determine the *GSTP1 Ala114Val* genotype.

GSTT1 genotype findings

It was identified that 85 (75%) patients had *GSTT1* gene deletion out of a group of 113. The result of electrophoresis which was used to determine the *GSTT1* genotype is displayed in Figure 5. GST isoenzymes of the patients enrolled for the study and their non-response to treatment were evaluated at Tables 1-4. Accordingly, distribution of isoenzymes and the rate of non-

response among 113 patients were identified as follows: 84 wild type (69%), 15 heterozygote (87%), 14 mutant(93%) for GST exon 6; and 59 wild type (73%), 44 heterozygote (75%), 10 mutant (%80) for GST exon 5. It was observed that there were 49 patients (76%) with *GSTM1* and 64 patients (73%) without *GSTM1*. Also 85 patients (74%) had *GSTT1* positive and 28 patients (75%) had *GSTT1* null genotype.



1st DNA marker corresponded to 100 bp, 2nd *GSTM1* genotype corresponded to 215 bp band; and *CYP1A1* corresponded to 312 bp band for the internal control, 3rd *GSTT1* genotype corresponded to 480 bp band; and *CYP1A1* corresponded to 312 bp band for the internal control, 4th *GSTM1* genotype corresponded to 215 bp band; and *CYP1A1* corresponded to 312 bp band for the internal control, 5th *GSTM1* and *GSTT1* deletion; and *CYP1A1* corresponded to 312 bp band for the internal control.

Figure 5: The result of gel electrophoresis which was used to determine the *GSTM1* and *GSTT1* genotypes.

Table 1: Possible risk factors in “non-response” to treatment.

<i>GSTP1</i> Exon6	Non-responder	Total number of patients	Prevalance (%)	OR	95 % CI
Wild type	58	84	69,0	-	-
Heterozygote	13	15	86,7	2,844	0,547-14,791
Mutant	13	14	92,9	9,757	1,002-95,024

Table 2: Possible risk factors in “non-response” to treatment.

<i>GSTP1</i> Exon5	Non-responder	Total number of patients	Prevalance (%)	OR	95 % CI
wild type	43	59	72,9	-	-
heterozygote	33	44	75,0	0,897	0,329-2,447
mutant	8	10	80,0	0,673	0,085-5,304

Table 3: Possible risk factors in “non-response” to treatment.

<i>GSTM1</i>	Non-responder	Total number of patients	Prevalance (%)	OR	95 % CI
No	47	64	73,4	-	-
Yes	37	49	75,5	1,132	0,435-2,946

Table 4: Possible risk factors in “non-response” to treatment.

<i>GSTT1</i>	Non-responder	Total number of patients	Prevalance (%)	OR	95 % CI
No	63	85	74,1	-	-
Yes	21	28	75,0	1,157	0,374-3,580

Table 5: Possible risk factors in “non-response” to treatment.

Cigarette (pack-year)	Non-responder	Total number of patients	Prevalance (%)	OR	95 % CI
0-19	11	15	73,3	-	-
20-39	28	34	82,4	1,663	0,354-7,815
40-59	12	19	63,2	0,466	0,085-2,565
60-79	17	22	77,3	1,369	0,278-6,749
80 and above	16	23	69,6	0,762	0,164-3,546

Table 6: Possible risk factors in “non-response” to treatment.

Alcohol consumption	Non-responder	Total number of patients	Prevalance (%)	OR	95 % CI
Non	56	78	71,8	-	-
Light	13	16	81,3	1,289	0,297-5,599
Medium	4	5	80,0	2,085	0,148-29,424
Heavy	11	14	78,6	1,974	0,457-8,531

Table 7: Possible risk factors in “non-response” to treatment.

Cancer type	Non-responder	Total number of patients	Prevalance (%)	OR	95 % CI
Small cell	16	32	50,0	-	-
Non-small cell	68	81	84,0	5,231	2,101-13,021

Smoking habits of the patients and the rate of their non-response to treatment were evaluated at Table 5. Accordingly, it was observed that 15 patients (73%), 34 patients (82%), 19 patients (63%), 23 patients (70%) and 23 patients (70%) smoked 0-19 boxes/year, 20-39 boxes/year, 40-59 boxes/year, 60-79 boxes/year and 80≥ boxes/year, respectively. Patients' alcohol ingestion and their non-response rate to treatment were evaluated at Table 6. It was determined that 78 (71%), 16 (81%), 5 (80%) and 14 patients (79%) were non-drinkers, light drinkers,

medium drinkers and heavy drinkers, respectively. Distribution of patients according to the cancer type and their non-response rate to treatment were evaluated at Table 7. It was determined that 32 (50%) and 84 (84%) patients had small-cell lung cancer and non-small cell lung cancer, respectively. Patients' alcohol ingestion was evaluated in accordance with their smoking habits and responses to treatment in Table 8. In table 9, smoking habits of the patients were evaluated via their response to treatment for *GSTP1* exon6 gene.

Table 8: Possible risk factors in “non-response” to treatment.

Alcohol	Cigarette (box-year)	Response		Total	Non-responder Prevalance (%)
		Yes	No		
Non	0-19	3	10	13	76,9
	20-39	5	17	22	77,3
	40-59	6	9	15	60,0
	60-79	4	10	14	71,4
	80 and above	4	10	14	71,4
Light	0-19	1	1	2	50,0
	20-39	1	6	7	85,7
	40-59	0	0	0	
	60-79	0	3	3	100,0
	80 and above	1	3	4	75,0
Medium	0-19	0	0	0	---
	20-39	0	2	2	100,0
	40-59	0	1	1	100,0
	60-79	0	0	0	---
	80 and above	1	1	2	50,0
Heavy	0-19	0	0	0	---
	20-39	0	3	3	100,0
	40-59	1	2	3	66,7
	60-79	1	4	5	80,0
	80 and above	1	2	3	66,7

Table 9: Possible risk factors in “non-response” to treatment.

Cigarette (box-year)	Ex6	Response		Total	Non-responder Prevalance (%)
		Yes	No		
0-19	w	3	8	11	72,7
	h	1	1	2	50,0
	m	0	2	2	100,0
20-39	w	6	18	24	75,0
	h	0	6	6	100,0
	m	0	4	4	100,0
40-59	w	6	7	13	53,8
	h	0	0	0	yok
	m	1	5	6	83,3
60-79	w	5	15	20	75,0
	h	0	1	1	100,0
	m	0	1	1	100,0
80 and above	w	6	10	16	62,5
	h	1	5	6	83,3
	m	0	1	1	100,0

Discussion

This study is conducted to search the effects of GSTM1, GSTT1 and GSTP1 isoenzymes of glutathione S-transferase enzymes, alcohol ingestion and smoking on response to treatment in lung carcinoma patients receiving platinum-based chemotherapy. GSTP1 is the most strongly expressed isoenzyme in the human lung. GSTM1 and GSTT1 isoenzymes are expressed in the lung, though lesser [22,23]. GSTP1 is known as to be the most expressed GST in non-small cell lung tumors [24]. It was determined that GSTP1 levels of drug resistant tumors were high [25]. Among these, non-small cell lung tumors are remarkable, in particular [1, 26-28]. It is argued that GSTP1 directly participates in the detoxification of platinum components and that it plays a significant role in both intrinsic and acquired resistance to platinum components [7,29]. However, there are studies demonstrating that platinum resistance is not associated with GSTP1 [30,31]. Similar contradictory results were presented for ovarian carcinoma [32,33]. One of the important reasons for not being able to demonstrate the correlation between GSTP1 expression and platinum components at any time, may be the polymorphism of *GSTP1* gene. It is well-known that *GSTP1* gene is polymorphic. In one of the two mutations of *GSTP1* gene (Ile 105 Val), a single nucleotide substitution (A-G) at position 313 results in replacing isoleucine with valine and thus enzyme activity diminishes. However, heterozygotes display medium-level enzyme activity.

Moreover, it is reported that GSTM1 and GSTT1 may be effective in the formation of several carcinomas, including lung carcinoma, and in response to treatments [34-36]. Since *GSTM1* and *GSTT1* genes are also polymorphic, lack of these genes results in loss of enzyme activities [37]. In this study, the responses to cisplatin based chemotherapy of homozygous and heterozygous patients with GSTP1 exon5 variant allele were observed to be like the responses of the patients carrying wild type genotype. GSTP1 develops resistance to etoposide, chlorambucil, melphalan and adriamycin as well as to cisplatin and carboplatin [9,38]. Therefore, the beneficial effect of GSTP1 exon5 variant allele cannot be attributed to a specific drug concerning treatment response, for instance on the response to cisplatin, but its effect can also be attributed to the combinations including these drugs concerning the response that may be received. However its benefit on the chemotherapy applied with drugs other than those mentioned (eg. 5-Fluorouracil) or with other combinations of drugs will remain undetermined until it is demonstrated that the mentioned drugs are detoxified by GSTP1. The reasons why results obtained in this study are different from those obtained on colon and breast cancers and leukemia may be that number of patients is less, different resistance mechanisms are dominant, different tumor types and chemotherapy regimens applied according to the cancer types. Furthermore, it was determined that *GSTP1*

genotypes who responded and did not respond to chemotherapy did not have a significant relation with age, gender and the phase of the disease.

The reason why *GSTM1* and *GSTT1* polymorphisms do not have effect on chemotherapy in non-small cell lung cancer patients may be that they are expressed in lung in small quantities. Similar findings were identified in colon cancer patients receiving platinum based treatment [39]. It is reported that the expressions of these enzymes are less in colon tumors as the case be in lung tumors and that on the other hand, GSTP1 is expressed more [40]. On the other hand, data is not sufficient about the predictive role of GSTs on the efficiency of cisplatin based drugs. It was demonstrated by using rats that GST Mu displayed resistance to cisplatin [41]. However, it was not displayed that these were the substrates of human GSTM1. Additionally, the role of GSTT1 in the detoxification of cisplatin is unknown. However, both of these two GSTs may function during the inactivation of lipid peroxides [42,43]. Thus, it may be considered that these GSTs may contribute to develop resistance by reducing the cytotoxic effects of alkylating agents through this method. However this study gives the impression that this case is not valid.

It is reported that in glioma cancer patients, *GSTM1* gene, together with *GSTP1* increases the responses to chemotherapy including nitrosourea alkylating agents [44]. However, in our study, doublet and triplet combinations of GSTM1, GSTP1 and GSTT1 genotypes did not demonstrate any effect on the response to chemotherapy as a whole. When the structure of relation between the patients' response or non-response to treatment and their gene isoenzymes was examined, it was observed that those who had GSTP1 exon 6 mutant genotypes responded to treatment 9 times more than those having GSTP1 exon wild type genes. Although it was observed that patients smoking 80 or more boxes a year responded to treatment, it is clear from the table that these patients' rate of having mutant or heterozygote genes is low. Thus, it is obvious that the evaluations to be conducted only according to the frequency of smoking will be mistaken. Under these circumstances, it is displayed that wild type genes have positive effect on the response to treatment. This results yields that it is important to evaluate the effect of combination of GST genotype, alcohol ingestion amount, smoking frequency on the response to treatment.

In conclusion, statistically significant relation could not be established between the the effects of GSTM1, GSTT1 and GSTP1 isoenzymes of glutathione S-transferase enzymes and ethyl alcohol ingestion and smoking on response to treatment in lung carcinoma patients receiving platinum-based chemotherapy. However, it is observed in terms of response to treatment that *GSTP1* Exon 6 gene's being wild type, low or little alcohol ingestion, being a non smoker or low smoking decrease the risk factor.

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