ORIGINAL ARTICLE

Eastern Journal of Medicine
East J Med 23(3): 160-167, 2018
DOI: 10.5505/ejm.2018.91885

Beta-fibrinogen 455 G/A and angiotensin converting enzyme ins/del polymorphisms in patients with lung cancer

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ABSTRACT

In our study we have investigated polymorphism due to base change occurring at -455 position of promoter region of β-fibrinogen synthesizing gene in lung cancer patients. In addition, we have also investigated insertion/deletion (I/D) polymorphism in intron 16 of Angiotensin Converting Enzyme (*ACE*) gene, located on chromosome 17q23. Aim of our study is to determine polymorphisms of β-fibrinogen -455 G/A and ACE I/D in patients with lung cancer and healthy control and to determine whether these polymorphisms contribute to the formation of lung cancer. In addition, we aimed to clarify whether β-fibrinogen -455 G/A polymorphism effect the level of fibrinogen synthesis in patients with lung cancer.

Samples in our study was obtained from 100 patients with lung cancer and 100 healthy volunteers. Plasma fibrinogen levels were measured with ELISA method. Polymorphism analyses were determined with PCR-reverse hybridization method.

Results of our study revealed that fibrinogen levels of patients with lung cancer were significantly higher than healthy control group ($p<0.01$). No difference was determined for β-fibrinogen -455 G/A and *ACE* I/D polymorphisms between patients with lung cancer and healthy control group. In both of the groups fibrinogen level was higher in AA genotype compared to GG and GA genotypes in both of the groups ($p<0.05$)

As a result, it can be stated that β-fibrinogen -455 G/A and *ACE* I/D polymorphisms are not related with formation and prognosis of lung cancer.

Key Words: Lung cancer, ACE I/D, β-fibrinogen -455 G/A, polymorphism

Introduction

Lung cancer is the most common cancer type in the World currently. According to Globocan 2012 data published by International Cancer Agency (IARC) 14.067.894 cancer cases were diagnosed worldwide and lung cancer formed 13% of those cases (1.824.701) (1). In Turkey, according to data by T.R. Ministry of Health, Administration of Public Health, Department of Cancer, lung cancer incidence is at 1st order in males and at 4th order in females (2). Lung cancer is divided into two groups histologically: non small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (3, 4). SCLC is the most aggressive with highest mortality rate and accounts 12-14% of all lung cancer cases (5). NSCLC is the most commonly encountered lung cancer type and constitutes approximately 80-83% of all cases diagnosed with lung cancer. NSCLC also divided to histological subtypes such as adenocarcinoma, squamous cell carcinoma, large-cell carcinoma and broncoalveolar carcinoma. Among those subtypes, adenocarcinoma is the most common (6). Formation of lung cancer is related with various environmental and genetic factors (7). Although patients diagnosed with lung cancer at similar stages and histological subtypes, they can have great differences in their prognosis. Those differences may be related with increase or decrease of some proteins (ex. proteins counterpart in coagulation) which are not directly related with cancer formation (8).

Fibrinogen is a glycoprotein synthesized in hepatocyte cells with a molecular weight of 350 KD (9). Fibrinogen is formed of two sets of alpha, beta and gamma polypeptide chains which are attached to each other with disulfide bridges (10). Thrombin transforms fibrinogen to water insoluble fibrin which has a role in coagulation, inflammatory response, fibrinolysis and wound healing (10). Three polypeptides forming fibrinogen is coded in q23-32 arm of 4th

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Received: 22.01.2018, **Accepted:** 02.04.2018

chromosome. Various polymorphisms were determined on gene performing fibrinogen synthesis. Among those polymorphisms, β chain polymorphisms are studies extensively since β chain is the time limiting step in adult fibrinogen synthesis (11). Nucleotide transition (-455G/A) at -455 position of the promoter region of β-fibrinogen gene increases plasma fibrinogen level (12). There are many studies revealing the relationship between high plasma fibrinogen concentration and angiogenesis, proliferation and metastasis of tumor cells (13). In studies concerning relationship between lung cancer and enhanced fibrinogen concentration, high plasma fibrinogen concentration is suggested as an important marker for tumor metastasis and patient prognosis (8, 14). However, no study was conducted investigating relation between lung cancer and β-fibrinogen -455 G/A gene polymorphism.

Angiotensin converting enzyme (ACE) is a zinc containing metallopeptidase and converts inactive dekapeptide angiotensin I to vasoactive angiotensin II. In addition ACE inactivates vasodilator brandikinin (15). The *ACE* gene is localized to chromosome 17q23 (16). Most common polymorphism in *ACE* gene is the presence (insertion, I) and the absence (deletion, D) of 287 base fragments in intron 16 (*ACE* I/D). *ACE* I/D polymorphism is directly related with plasma ACE concentration and it is found that tissue and plasma ACE concentrations increase two fold in DD genotype carriers compared to genotype II (16, 17).

Aim of our study is to determine ACE I/D and β-fibrinogen -455 G/A polymorphisms in patients with lung cancer and healthy control group and provide any relationship between lung cancer and these polymorphisms.

Materials and Methods

Clinical Parameters of Patients: 100 patients who get inpatient treatment and diagnosed with lung cancer (Male:78, Female:22) was included into our study who administered to Atatürk University Faculty of Medicine Research Hospital, Department of Chest Diseases and get lung cancer diagnose after pathological and radiological tests. Lung cancer classification of this patient group was performed by Atatürk University Faculty of Medicine Department of Pathology. State of smoking, gender and ages of patients were obtained from patient files. 100 controls (Male:68, Female:34) in our study, were chosen among healthy volunteers without any chronic disease and their age group (62.08±9.34) was chosen similar compared to patient group (63.66±8.1) (Table 1).

Tissue Procurement: 6 ml of blood samples obtained from patient and healthy volunteers are divided into EDTA containing tubes (3 ml) and sodium citrate containing tubes (3 ml) and following aliquot stored at -80 C^0 until study.

Analyses of Polymorphisms: For analysis of β-fibrinogen -455 G/A and *ACE* I/D gene polymorphisms, venous blood samples were withdrawn into EDTA containing tubes and polymorphism analysis were done by using CVD StripAssay A kit (ViennaLab Diagnostics, Austria). Such analysis processes were performed in three steps including, DNA isolation, amplification with polymerase chain reaction (PCR) using biotinylated primers and reverse hybridization of amplification end products. Those procedures were conducted according to protocols of commercially obtained kits. In β-fibrinogen -455G/A polymorphism; wild type bearing G/G alleles, heterozygote bearing G/A alleles and homozygote bearing A/A alleles. In ACE I/D gene polymorphisms definitions were made as follows; I/I wild type, I/D heterozygote and D/D homozygote (Figure 1).

ELISA: Fibrinogen analysis in plasma samples were conducted by using Assaypro Human Fibrinogen ELISA (Cat No: EF1040-1) kit. Measurements were conducted in microplate reader at 450 nm wavelength (Bio-Tek PowerWave XS). For the kit intra-assay variation coefficient was 5.1%, inter-assay variation coefficient was 7.5% and minimum detectable value was 0.16 μg/ml. Results were presented as g/l. In addition according to instructions given in kit, 1.5-4 g/l range was defined as normal fibrinogen concentration whereas >4 g/l was defined as hyperfibrinogenemia.

Statistical Analysis: Statistical analysis of data was performed by using IBM-SPSS 19.0 statistical program. Distribution of variables was checked via Kolmogorov-Smirnov test. Normal distribution of numeric variables were found and sample-T test was used for statistical analysis of difference between variables in two groups. For assessment of difference of variables in three and more groups One-way-ANOVA test was used. For the analysis of categorical data X^2 (Chi-square) test was used. $P<0.05$ value was accepted as statistically significant.

Table 1. Demographic features of patients with lung cancer and health control groups

	Patients with Lung Cancer Group N=100	Healthy Control Group N=100	p value
Age (years) (Mean±SD)	63.66±8.1	62.08±9.34	0.204
Status of smoking			
Non-smoker	6 (6%)	27 (27%)	0.01
Smoker	94 (94%)	73(73%)	0.01
Gender			
Male	78 (78%)	66 (66%)	0.59
Female	22 (22%)	34 (34%)	0.59
Classification			
SCLC	24 (24%)		
NSCLC	76 (76%)		

Table 2. β-fibrinogen -455 G/A and *ACE* I/D gene polymorphism distributions of in patients with lung cancer and healthy control groups

		Patient Group	Healthy Control	OR(Low-High)
β-Fibrinogen Gene Polymorphism -455G/A	-455GG n(%)	59(59%)	59(59%)	1.00(0.569-1.757)
	-455GA n(%)	32(32%)	33(33%)	0.955(0.529-1.727
	-455AA n(%)	9(9%)	8(8%)	0.879(0.325-2.379)
	Allele			
	A allele	0.250	0.245	1.130(0.694-1.841)
	G allele	0.750	0.755	0.961(0.821-1.126
	D/D	46 (46%)	42(42%)	1.176(0.673-2.057)
	I/I	23(23%)	18(18%)	1.161(0.682-2.715)
ACE Gene Polymorphism I/D	I/D	31(31%)	40(40%)	0.674(0.376-1.207)
	Allele			
	D allele	0.615	0.620	0.998(0.805-1.242)
	I allele	0.385	0.380	0.994(0.702-1.425)

OR: Odds ratio

Results

Statistical analysis of age, state of smoking and gender data of patients with lung cancer and healthy control group are summarized in Table 1. There were no statistically significant difference between gender and age values of patients with lung cancer and healthy control group. Fibrinogen values observed in lung cancer patient group were significantly higher compared to control (5.12±0.72, 3.62±1.05, respectively $p<0.01$) (Figure 2). When β-fibrinogen -455 G/A and *ACE* I/D gene polymorphisms in patient and control groups were analyzed no statistically significant difference was observed for GG, AA and GA genotype distributions (Table 2). No statistical difference was detected between patient and control group for frequency of A allele ($p=0.25$ and $p=0.245$ respectively). Same situation was also valid for G allele frequency ($p=0.75$ and $p=0.755$ respectively). No statistically significant difference was observed for *ACE* I/D gene polymorphism between patient and control groups. In addition no statistical difference was observed for D allele frequencies and I allele frequencies (Table 2). When patients with lung cancer are divided into two groups as SCLC and NSCLC and β-fibrinogen -455 G/A and ACE I/D gene polymorphisms are evaluated, patients who have AA genotype are found 66.6% in SCLC

Table 3. -455 G/A and ACE I/D gene polymorphism distributions of β-Fibrinogen in patients with SCLC and NSCLC

Classification in Patients with Lung Cancer		
β-Fibrinogen Gene Polymorphism	SCLC	NSCLC
	N=24	N=76
-455GG n(%)	10 (19%)**	49(71%)
-455GA n(%)	8 (25%)**	24 (75%)
-455AA n(%)	6 (66.6%)*	3 (33.7%)
Allele Frequency		
A allele	0.416**	0.197
G allele	0.583**	0.802
ACE I/D Gene Polymorphism		
D/D	10 (21.7%)**	36 (78.3%)
I/I	6 (26%)**	17 (74%)
I/D	8 (25.8%.)**	23 (74.2%)
Allele Frequency		
D allele	0.583*	0.605
I allele	0.417*	0.395

Percentages presented in table is not for intragroup but within allele percentages.
*: statistically significant difference when compared to NSCLC group $p<0.05$.
**: statistically significant difference when compare to NSCLC group $p<0.001$.

whereas 33.7% in NSCLC. GG genotype bearers were found 19% in SCLC and 71% in NSCLC patients. GA genotype bearers were 25% SCLC patients whereas 75 % of them were NSCLC patients. *ACE* D/D genotypes constituted 21.7% in SCLC patients whereas 78.3% in NSCLC patients. *ACE* I/I genotypes accounted 26% in SCLC patients whereas 47% in NSCLC patients. Patients who have *ACE* I/D genotype are found 25.8% in SCLC patients and 74.2% in NSCLC patients. Distribution of β-fibrinogen -455 G/A and *ACE* I/D gene polymorphisms in SCLC and NSCLC patients are summarized in Table 3.

When fibrinogen concentrations were evaluated according to allele distribution in patients with lung cancer, AA genotype lung cancer patients are statistically significantly higher compared to patients with GG and GA genotypes (6.66±0.35, 5.02±0.53 and 4.88±0.57 respectively, $p<0.001$) (Figure 3). In healthy control group also, AA genotype have statistically significantly higher fibrinogen levels compared to fibrinogen levels of lung cancer patients bearing GG and GA genotypes (5.92±0.31, 3.56±0.91 and 3.17±0.58 respectively, $p<0.001$) (Figure 4). When patients with lung cancer are divided into two groups as NSCLC and SCLC and assessed for fibrinogen values no statistically significant difference was detected (5.36±1.14 and 5.04±0.81 respectively, $p>0.05$) (Figure 5).

Discussion

Lung cancer is a type of cancer with highest morbidity and mortality rates worldwide (18). Although lung cancer is directly related with tobacco and tobacco products or with exposure, genetic factors have an important role (19). Those differences may be related with increase or decrease of some proteins (ex. proteins counterpart in coagulation) which are not directly related with cancer formation. It has been known for 150 years that haemostatic factors have a role in cancer etiopathogenesis (20). Various haemostatic anomalies such as intravascular coagulation, hemorrhagic events and migratory tromboflebitis occur in cancer patients. Such haemostatic anomalies are placed in second order as cause of death (21,22). Many coagulation abnormalities are reported for cancer patients. Augmented fibrinogen activity is among them (23). Studies concerning fibrinogen which is a coagulation factor and cancer, augmented fibrinogen activity was found to affect tumor cell growth, progression and metastasis (23). Hyperfibrinogenemia is found related with colorectal (24), cervical (25), over (26), esophagus (27) and pancreas (28) cancer types and found to increase in such cancer types. Guo et al (28) found an increase in serum fibrinogen level in pancreas cancer patients and also showed increased risk for distal metastasis in patients with high fibrinogen level after follow-up those patients. There

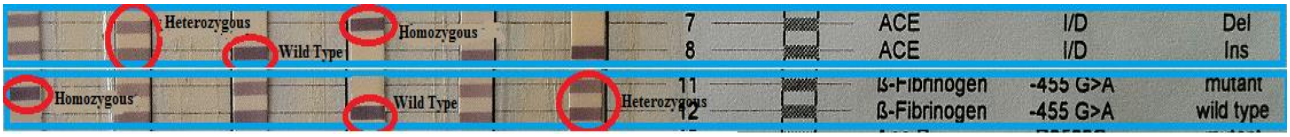

Fig. 1. Bands obtained after reverse-hybridization of samples and evaluation of these bands

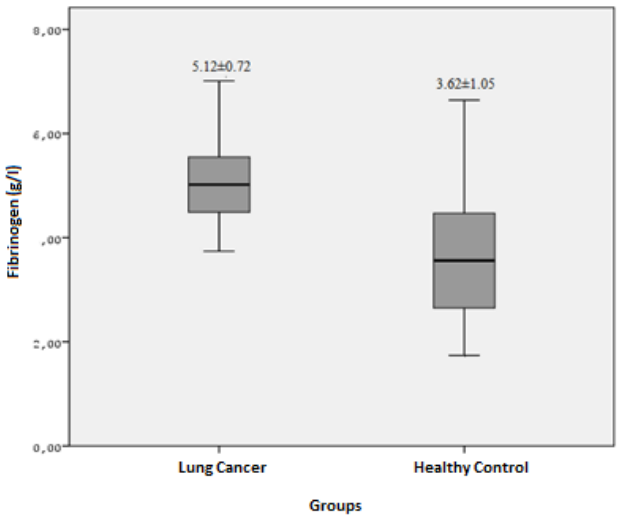

Fig. 2. Box plot presentation of fibrinogen levels of patients with lung cancer and healthy control groups. Mean±SD values are shown

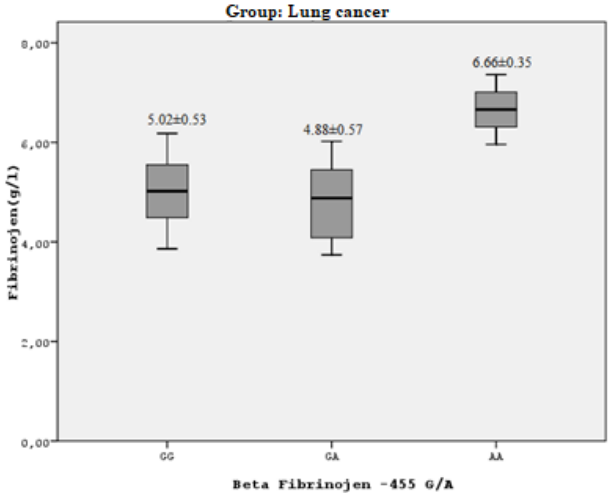

Fig. 3. Box plot presentation of fibrinogen levels of patients with lung cancer group. Mean±SD values are shown

are numerous studies concerning relationship between lung cancer and fibrinogen. Lu et al. (29) determined serum fibrinogen levels in 21 lung cancer, 5 hepatic cancer, 8 stomach cancer, 16 colon cancer and a total of 139 cases diagnosed with cancer and compared with healthy control group. As a result, they found that the fibrinogen levels of patients with lung cancer were higher than both control and the mean of all cancer patients.

Result obtained in our study was supporting results of previous studies. Fibrinogen levels in patients with lung cancer were statistically significantly higher compared to observed values in healthy control group. In addition, when we divided our patient group into two groups as NSCLC and SCLC and checked for fibrinogen levels, hyperfibrinogenemia was observed in both of the groups. Fibrinogen levels in NSCLC patients were higher than SCLC patients however we observed that this rise was not statistically significant. Many physiological and pathological reasons may exist for this rise. However, we couldn't encounter any literature focusing on this rise in fibrinogen concentration in lung cancer patients is related with genetic origin in our literature search. In our study we have investigated -455 G/A polymorphism since A allele causing this polymorphism is found about 20% of adult population and affects fibrinogen concentration directly (30). In the case of A allele homozygosity which is observed in about 1-5% of population, fibrinogen levels were augmented, whereas in G allele homozygosity or heterozygosity no difference was observed in fibrinogen concentration in our study. Results obtained in our study are in consistence with literature records. In both patients with lung cancer and healthy control group, group bearing AA genotype had statistically significantly higher fibrinogen levels than group bearing GG and GA genotypes.

There are two studies focusing on -148 C/T and gama-10034 C/T polymorphisms in colorectal cancer patients. Vossen et al. (31) in their study, investigated protrombine G20210A, PAI-1 4G/5G, MTHFR 677 C/T, factor XIII Val34Leu and gamma-fibrinogen 10034 C/T polymorphisms in 1801 patients with colorectal disease and 1853 healthy control group. As a result, they have detected, 978 CC allele bearing, 656 CT allele bearing and 103 TT allele bearing colorectal cancer patients were determined according to their obtained gamma-fibrinogen 10034 C/T. Finally, they have found no relationship between colorectal cancer risk and evaluated polymorphisms including gamma-fibrinogen 10034 C/T polymorphism. Wang et al. (32) investigated β-fibrinogen -144C/T polymorphism in patients with colorectal cancer and found no difference between cancer and healthy group in T allele frequency which causes increase in fibrinogen concentration. In our study in 59 of patients (59%) with lung cancer GG, in 32 (32%) GA and in 9 (9%) AA genotypes were found. Similarly in analysis performed on healthy group in 59 individuals

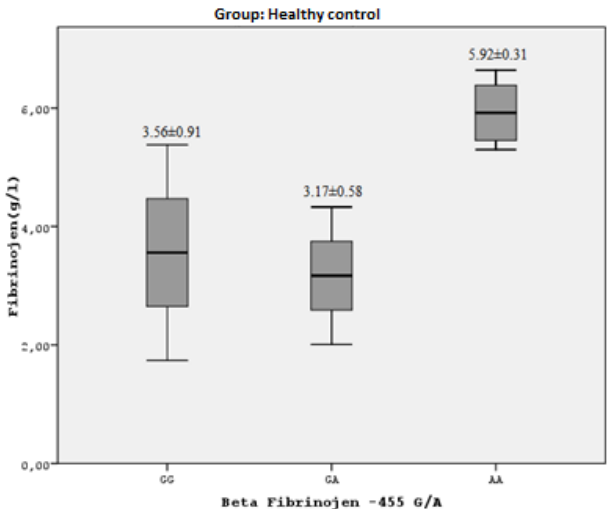

Fig. 4. Box plot presentation of fibrinogen levels of healthy control group. Mean±SD values are shown

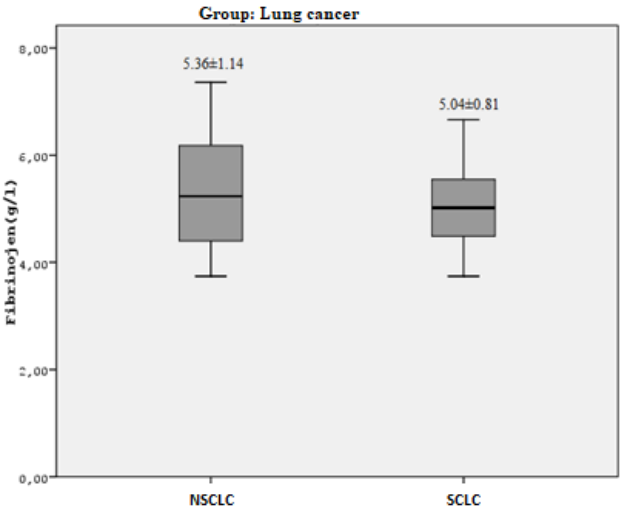

Fig. 5. Box plot presentation of fibrinogen levels of patients with NSCLC and SCLC. Mean±SD values are shown

(59%) GG, in 33 (33%) GA and in 8 (8%) AA genotypes were determined. No difference was found between patients with lung cancer and healthy control group for β-fibrinogen -455G/A polymorphism. When patients with lung cancer were grouped according to GG, GA and AA genotypes and assessed for their fibrinogen levels, group bearing AA genotype had fibrinogen level significantly higher than two other groups but fibrinogen level of all groups were higher than 4 g/l (hyperfibrinogenemia). In the healthy control group, group bearing AA genotype had fibrinogen level higher than two other groups however group bearing GG and GA genotypes had fibrinogen mean lower than 4 g/L. According to these results, β-fibrinogen -455G/A polymorphism can be considered as nonrelated with lung cancer formation and prognosis. In addition, it can be stated that it is nonrelated with rise of fibrinogen levels in patients with lung cancer.

Patients with lung cancer were divided into groups with NSCLC and SCLC histologically and β-fibrinogen -455 G/A polymorphism was investigated. GG genotype detected in 59% of patients with lung cancer was found 29% in SCLC and 71% in NSCLC. In addition, GA genotype detected in patients with lung cancer was found 25% in SCLC and 75% in NSCLC. Also AA genotype detected in patient group was found 66.6% in SCLC and 33.7% in NSCLC. When frequencies of A and G alleles were investigated, A allele frequency was found statistically significantly higher in SCLC. In SCLC patients with high A allele frequency, fibrinogen levels were found higher than NSCLC patients, but it was not statistically significant. According to this data, A allele was suggested to have a role in prognosis of SCLC which is a more aggressive form of lung cancer with lower life expectance however a study with a wider population is needed to clearly interpret this situation.

ACE is an important enzyme found in renin-angiotensin system (RAS) (33). This enzyme has an important role in vascular homestasis modulation, inflammation, and angiogenesis (34). ACE is synthesized in various tissues and mainly in lung, liver, heart and testis (35). There are studies showing detoriation of ACE activity in some cancer types (36-38). ACE I/D polymorphism is evaluated in patients with lung cancer by many researchers and contradictory results were obtained. Nacak et al.(39) investigated *ACE* I/D polymorphism in 125 lung cancer patients and in 165 healthy control group, and found higher I allele frequency in patient group. According to this result, Nacak et al. Have indicated that I allele may have a role in formation of lung cancer and as an indicator for genetic susceptibility for lung cancer. Yaren et al. (40) found a higher D allele frequency in NSCLC patients and mentioned about a possibility of anemia caused by D allele in those patients. Cheon et al. (41) investigated *ACE* I/D polymorphism in 218 patients with lung disease and in 121 healthy control group and found no statistically significant difference between allele frequencies and suggested no relationship between this gene polymorphism and lung cancer formation. There were efforts to clarify those contradictory results with recently performed meta-analyses. Cheng et al. (42) in their meta-analyses, included 203 studies dealing with *ACE* I/D gen polymorphism and lung cancer, and compared a total of 1612 patients with lung cancer and 1442 healthy control group. Following this meta-analysis, no relationship between *ACE* I/D gene polymorphism was found. Results obtained in our study were in parallel with result

obtained in this meta-analysis. In the lung cancer patient group we have identified D/D genotype in 46 (46%), I/I genotype in 23 (23%) and I/D genotype in 31 (31%) patients. In healthy control group we have identified D/D genotype in 42 (42%), I/I genotype in 18 (18%) and I/D genotype in 40 (40%) person. When two groups were compared for D/D, I/D and I/I genotype distributions no significant difference was observed. In addition when I and D allele frequencies were evaluated no statistical difference was found. However, when distribution of such genotypes among SCLC and NSCLC patients were assessed DD genotype determined in 46 patients were found 21.7% in SCLC and 78.3% were in NSCLC. ID genotype determined in 31 patients were found 25.8% in SCLC and 74.2% in NSCLC, II genotype determined in 23 patients were found 26% in SCLC and 74% in NSCLC. No statistical difference was found in D and I allele frequencies in NSCLC and SCLC patients. Our results reveal that, there exists no relation between *ACE I*/D polymorphism and lung cancer formation.

As a result, it is possible to state that β-fibrinogen -455 G/A and *ACE* I/D polymorphisms are not related with formation and prognosis of lung cancer and no genetic susceptibility to lung cancer is found in persons bearing these polymorphisms. In addition increase in fibrinogen level in patients with lung cancer is not probably caused by genetic origin.

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