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Original Article / Özgün Makale

Role of vitamin D binding protein (VDBP) gene polymorphisms in lung cancer

Vitamin D bağlayan protein gen polimorfizmlerinin akciğer kanserindeki rolü

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ABSTRACT

Background: This study aims to investigate the role of different alleles of GC gene in the etiology of lung cancer.

Methods: The study included 77 patients with lung cancer (73 males, 4 females; mean age 59.6±9.2 years; range 21 to 72 years) and 25 healthy individuals (21 males, 4 females; mean age 47.3±5.4 years; range 23 to 61 years). Polymorphisms in vitamin D binding protein gene of all participants were examined by polymerase chain reaction-restriction fragment length polymorphism method.

Results: Of the patients, the genotype 1S-2 was found in 32.4%, 1F-1S in 28.6%, 1S-1S in 28.6%, 1F-2 in 7.8% and 1F-1F in 2.6%, while the genotype frequencies in control group were 28%, 24%, 36%, 4% and 8%, respectively (p=0.35).

Conclusion: We did not detect any relationship between vitamin D binding protein gene polymorphisms and lung cancer.

Keywords: Lung cancer; polymorphism; vitamin D binding protein.

ÖZ

Amaç: Bu çalışmada GC geninin farklı alellerinin akciğer kanseri etiyolojisindeki rolü araştırıldı.

Çalışma planı: Çalışmaya akciğer kanserli 77 hasta (73 erkek, 4 kadın; ort. yaş 59.6±9.2 yıl; dağılım 21-72 yıl) ve 25 sağlıklı birey (21 erkek, 4 kadın; ort. yaş 47.3±5.4 yıl; dağılım 23-61 yıl) dahil edildi. Tüm katılımcıların vitamin D bağlayan protein genindeki polimorfizmler polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi yöntemi ile incelendi.

Bulgular: Hastaların %32.4'ünde 1S-2, %28.6'sında 1F-1S, %28.6'sında 1S-1S, %7.8'inde 1F-2 ve %2.6'sında 1F-1F genotipi bulunurken kontrol grubunda genotip frekansı sırasıyla %28, %24, %36, %4 ve %8 idi (p=0.35).

Sonuç: Vitamin D bağlayan protein gen polimorfizmleri ve akciğer kanseri arasında herhangi bir ilişki saptanmadı.

Anahtar sözcükler: Akciğer kanseri; polimorfizm; vitamin D bağlayan protein.

Lung cancer is one of most prominent cancers both seen in men and women frequently and causing death. Additionally, in both genders, the risk of getting cancer increases with age. While it is split into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) categories based on lung cancer pathology, NSCLC comprises 80% of all lung cancer cases.[1] Although epidemiologic studies suggest that tobacco use is at the top of the list of factors causing lung cancer, it was reported that the 10% of individuals getting lung cancer are nonsmokers.[2,3] This shows that, in addition to tobacco use, genetic, epigenetic and other environmental factors have an effect on formation and development of lung cancer.

Vitamin D binding protein (VDBP) (also known as GC or Gc-globulin) is a protein weighing 58 kDa which is excreted by liver. It is found in the plasma, cerebrospinal fluid and on the membranes of B lymphocytes and functions through interacting with surface immunoglobulins. Some of the known functions of Vitamin D binding protein are activation of macrophages (macrophage activating factor-MAF) and taking role in neutrophilic reactions. In addition

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to binding to and carrying metabolites, Vitamin D has an important function like affecting the density of inflammatory reaction.[4] Additionally, it blocks the entrance of monomeric G actin protein into blood flow by binding to it. So, by preventing the filament formation of G-actin, it prevents vein and tissue damage.[5] GC gene which is the gene responsible of encoding the Vitamin D binding protein, is located in q11-q13 area of 4th chromosome in humans, and 3 isoforms of this gene (1F, 1S and 2) are formed based on single nucleotide changes in 11th exon (SNP, rs4588 (420 ACG>AAG (Thr>Lys) and rs7041 (416 GAT>GAG (Asp>Glu).[6]

It is thought that cancer development is accelerated due to insufficient activation of macrophages in advanced cancer cases. It is considered that loss of macrophage activation which is one of the major functions of Vitamin D binding protein, has an important role in carcinogenesis.[7] Although there are several studies in the recent years providing evidence that VDBP takes role in cardiovascular diseases, autoimmune disorders, diabetes, asthma, Alzheimer's disease and tuberculosis development and several cancers, the available data contradicts with each other.[7-10]

Therefore, in the present study, we aimed to investigate the role of the different alleles of GC gene in lung cancer etiology.

PATIENTS AND METHODS

GC genotypes were determined by drawing blood from 77 patients diagnosed by lung cancer (73 males, 4 females; mean age 59.6±9.2 years; range, 21-72 years) and 25 healthy individuals who admitted to Istanbul University Cerrahpaşa Faculty of Medicine Department of Chest Diseases. Following DNA isolation from blood samples taken from patients and healthy individuals using standard protocols, genotyping was performed by using polymerase chain reaction- restriction fragments length polymorphism method (PCR-RFLP).

Following PCR amplification of the samples (Table 1), the amplicons were fragmented with Hae III and Sty I restriction endonucleases (Fermentas, Waltham, Massachusetts, USA) and incubated at 37°C overnight and visualized under UV light (Vilber Lourmat, Cedex, France) after running the samples on 3% agarose gel electrophoresis at 120 V for 30 min. The primers sequence specific to GC gene are as follows: 5' TAA TGA GCA AAT GAA AGA AG 3' for forward and 5' AAT CAC AGT AAA GAG GT 3' for reverse. The polymerase reaction conditions are given in Table 1. The final product of PCR was 388 bp long and the allele type was determined as 1S when the product was fragmented as 295 bp and 93 bp when processed with HaeIII and as 2 when the product was fragmented as 304 and 84 bp when processed with StyI enzyme. In case of both enzymes did not fragment the PCR product, the allele type was determined as 1F.

The study protocol was approved by Istanbul University, Faculty of Medicine, Ethics Committee. Patients were informed about the procedure and a written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Genotype and allele frequencies are analyzed by IBM-SPSS program version 21.0 (IBM Corp. Released 2012. Armonk, NY, USA) with Chi square test and it is evaluated as $p<0.05$ significant.

RESULTS

Genotyping was performed in 77 patients with non small cell lung cancer and 25 healthy control subjects with PCR-RFLP method to investigate the effects of vitamin D binding protein gene on lung cancer.

The allele types of the patients and control subjects (1F, 1S and 2) and their relation to lung cancer were

Table 1. Polymerase chain reaction conditions and program

Polymerase chain reaction conditions	Polymerase chain reaction program	
2.5 mM $MgCl_2$	94°C 10 min	
1x Buffer (50 mM KCl, 20 mM Tris-HCl (Ph: 8.3, 20 μg/mL BSA)	94°C 30 sec	30 cycles
200 μM dNTP	56°C 30 sec	
10 pmol each primer	72°C 30 sec	
2 U Taq polymerase	72°C 5 min	
50 ng genomic DNA		

KCl: Potassium chloride; Tris-HCl: Tris Hydrochloride; BSA: Bovine serum albumin; dNTP: Deoxy nucleotide triphosphate; DNA: Deoxyribonucleic acid.

Table 2. Clinopathologic data of patients and control group

	Patient group		Control group	
	n	%	n	%
Pathology				
Adenocarcinoma	18	23.4	-	-
Epidermoid	18	23.4	-	-
Other	11	14.3	-	-
Uncertain	30	38.9	-	-
Stage				
1-2b	5	6.5	-	-
3 and over	40	51.9	-	-
Uncertain	32	41.6	-	-
Smoking				
0-30 package/year	22	28.6	17	68
30-60 package/year	30	38.9	8	32
≥60 package/year	25	32.5	-	-
Age				
<50	9	11.7	12	48
≥51	44	57.1	13	52
Uncertain	24	31.2	-	-

Table 3. Genotype distribution in patient and control groups

	Patient group		Control group		
Genotype	n	%	n	%	*p*
1S-1S	22	28.6	9	36	0.35
1F-1S	22	28.6	6	24	
1F-1F	2	2.6	2	8	
1S-2	25	32.4	7	28	
1F-2	6	7.8	1	4	
2-2	-	-	-	-	

Table 4. Comparison of allele frequencies and effects on lung cancer

Allele frequencies	Patient group		Control group		
	n	%	n	%	*p*
1S	91	59.1	31	62	0.81
1F	32	20.8	11	22	
2	31	20.1	8	16	

determined after restriction fragmentation with HaeIII and StyI. 6.5% of patients (n=5) were diagnosed as early stage NSCLC and 51.9% of them (n=40) were diagnosed as advanced NSCLC (Table 2). All patients were smokers. At the end of the study, it was seen that 32.4% of patients (n=25) had 1S-2, 28.6% of them (n=22) had 1F-1S, 28.6% of them (n=22) had 1S-1S, 7.8% of them (n=6) had 1F-2, 2.6% of them (n=2) had 1F-1F genotype. In the control group, it was seen that 28% of patients (n=7) had 1S-2, 24% of them (n=6) had 1F-1S, 36% of them (n=9) had 1S-1S, 4% of them (n=1) had 1F-2, 8% of them (n=2) had 1F-1F genotype. No 2-2 genotype was encountered in patient or control groups. No significant difference was found when patient and control groups were compared with regards to genotype (p=0.35) (Table 3). Both patient and control groups were distributed properly according to Hardy-Weinberg equilibrium (For patient group χ^2: 50.24, p=0.057 and for control group χ^2: 16.04, p=0.48). In lung cancer group, the allele frequencies of 1S, 1F ve 2 alleles were found as 59.1%, 20.8% and 20.1%, respectively. It was determined that allele frequencies were similar to that of patient group and they were 62%, 22% and 16%, respectively. No statistically significant difference was found between two groups (p=0.811) (Table 4). Relative odds ratio and 95% confidence interval values related to alleles are given in Table 5. No statistically significant values were obtained as regards to the fact that any allele had a providing predisposition or protective effect against lung cancer (p>0.05). No significant difference was found when genotypes were compared based on patient age, tobacco use, phase and pathology (p=0.68, p=0.53, p=0.76 and p=0.69, respectively).

Table 5. Relative probability rate and genotype distribution and frequencies at 95% confidence interval

Genotype	Relative probability rate	95% Confidence interval	*p*
1F-1F	1		
1S-1S	0.27	0.23-1.72	0.25
1F-1S	0.40	0.25-1.92	0.57
1S-2	0.28	0.23-1.73	0.25
1F-2	0.16	0.20-1.62	0.49
2-2	-	-	-

This value could not be calculated as 2-2 genotype was not able to be seen.

DISCUSSION

Vitamin D system is one of the systems which is active in regulation of several biological pathways among which cellular proliferation and differentiation are the primary ones. When vitamin D, which is not a real vitamin but a precursor of the hormone called calcitriol, is activated through CYP24A1 (24-hydroxylase) and CYP27B1 (1, α-hydroxylase) enzymes, it is bound to its specific receptor (Vitamin D Receptor-VDR) and acts as a transcription factor. Even though CYP24A1 causes the depletion of Vitamin D by degrading it, it is VDBP which determines the level of activated vitamin D in the circulation. Although VDBP, a glycated globulin, takes role in carrying of vitamin D and circulation of its metabolites, since it is a member of the scavenger system, it has importance in carcinogenesis by functioning in removal of hazardous elements occurring after tissue or cellular damage and prevention of formation of F-actin network. At the same time, vitamin D binding protein has a great importance in carcinogenesis since it takes role in macrophage activation and neutrophil chemotaxis.[11] For this reason, the relationship between the plasma level of VDBP and cancer risk were investigated extensively in a lot of studies in the recent years.

Vitamin D binding protein, whose main function is to carry vitamin D in the organism by binding to it, also functions in regulation of inflammatory response as a response to environmental factors. However, one of its main functions is to carry vitamin D to the lungs. Therefore, VDBP has a particular importance as regards to lung diseases. The studies carried out with epidemiologic and animal models show that activated vitamin D metabolites (serum $1,25(OH)_2D$) prevent the development and metastasis of lung cancer.[12] Besides, it is thought that VDBP protein might be functional in disease like chronic obstructive respiratory disease (CORD) and tuberculosis, due to the fact that it is found in bronchoalveolar lavage fluid.[13] One of the most important factors in lung pathogenesis is tobacco use. A lot of toxic and carcinogenic elements in found in cigarette smoke causes loss of normal physiological functionality and health problems by interacting with DNA, proteins and lipids. Vitamin D binding protein which was demonstrated as related to lung pathogenesis is one of the proteins which will be affected by these potential damages of cigarette. The gene encoding VDBP is a polymorphic gene and the polymorphisms causing changes in 416th and 420th amino acids of this protein has an impact on the functionality of the protein. Since macrophages will not be sufficiently active due to the loss of functionality of the protein, immune system might fall short in annihilating the cancerous cells. Particularly, GC2 allele is formed as a result of the change in 420th ending with lysine coding. In the studies carried out, it was noted that allele 2 had less macrophage activation feature and as a result of this the individuals having allele 2 had less macrophage functionality. While there are several studies carried out until today which suggests that the polymorphisms (rs7041 and rs4588) in the gene coding VDBP are related to the lung,[14] breast[15] and prostate[16] cancers, there are also studies stating that there is no relationship between GC polymorphisms and prostate,[17] colorectal cancer,[18,19] basal cell carcinoma[20] and melanoma (only rs7041 was examined).[21] The number of studies carried out until today in the literature investigating the relationship between VDBP polymorphism and lung cancer is very limited. In the study carried out by Maneechay et al.,[14] it was demonstrated that rs7041 (416 GAT>GAG (Asp>Glu) polymorphisms had a relationship with lung cancer, 61 of 113 patients had TG genotype and this was related to lung cancer ($p=0.037$). When single nucleotide polymorphisms in rs7041 and rs4588 were investigated in one combined, it was seen that having genotype 1F-2 (TT-CA) had a significant protective effect against lung cancer ($p=0.014$). However, in the same study, no relationship was found between both genotypes and colorectal and breast cancer.[14] In our study, the number of individuals having 1F-2 genotype was constituting 7.8% of the patient group and 4% of control group, respectively. When considered from this point of view, no relationship was found between these genotypes and lung cancer. The difference between them might stem from the study groups' racial differences. Except this study, there is no study showing the relationship between GC alleles and lung cancer, the number of studies showing the effect of 1S and 1F alleles on lung cancer is very limited as well.[22] In our previous study which we investigated the VDBP gene polymorphisms in COPD patients, we showed that 1F and 2 alleles are not related to COPD development or prevention, but having 1S-1S genotype might be related to COPD etiology,[23] on the other hand, in this study we could not find any relationship between lung cancer and GC polymorphisms. In a study carried out in white people, it was shown that the 1S, 1F and 2 allele frequencies were 0.56, 0.16 and 0.28 respectively.[24] The results we obtained in our study supports these data as well. However, we could not identify any significant relationship between 1S, 1F and 2 alleles and lung cancer. The results obtained from the studies carried out before which are especially showing the relationship between cancer

and VDBP gene in Asian people, might stem from the racial differences to a large extent. Additionally, the low number of subjects in our study makes it difficult to reach sufficient statistically significance.

In conclusion, it was demonstrated that there is no difference between vitamin D binding protein genotype distribution between lung cancer patients and healthy control group, anyone of the VDBP gene allele types does not cause any risk factor for lung cancer, in a similar way, anyone of the VDBP gene allele types does not provide any protection against lung cancer.

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