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Endothelin-1 Gene Polymorphism in Chronic Obstructive Pulmonary Disease: A Case-Control Study

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Abstract: In the current studies there being a high ratio of Endotelin-1 (ET-1) in BAL liquid of Chronic Obstructive Pulmonary Disease (COPD) cases made researchers think ET-1 may have an important role in pathogenesis of COPD. Our study group does research on the relation of COPD with single nucleotide gene polymorphism at ET-1 gene. This prospective case-control study included 87 smokers with COPD and 89 smokers but not COPD. We investigated the density of single nucleotide gene polymorphism (+134 insA/delA) in the ET-1 gene in cases. Allele ratio and genotype distribution, distribution amongst three genotype (3A3A, 3A4A, 4A4A) in the COPD patient and control group was analyzed. In this study, for endothelin gene -3A/-4A (-138 insertion/deletion) polymorphism analysis, polymerase chain reaction-restriction fragment length polymorphism method was used. In comparison with the control group, the COPD group has higher ratio of ET-1 gene (+134 insA/delA) polymorphism ($p<0.001$). Endotelin-1 gene polymorphism (+134 insA/delA) significantly increased in the smokers with COPD than control group ($p<0.013$). Endothelin-1 gene polymorphism may be a predictive genetic factor of COPD. ©2023 NTMS.

Keywords: Endothelin-1; Chronic Obstructive Pulmonary Disease; Gene Polymorphism.

1. Introduction

With population aging, tobacco use and other risks, chronic respiratory diseases have become an increasingly important cause of morbidity and mortality. Chronic obstructive pulmonary disease (COPD) is an increasingly important cause of death worldwide [1, 2]. In 2002, COPD was the eleventh cause of disability adjusted life years (DALYs); It is expected to be the seventh leading cause by 2030 [3].

Significant differences in onset, progression, and lung function between populations at different life stages limit understanding of the causes that predispose to COPD [2, 4]. However, tobacco use and air pollution and occupational pollutants have been reported as primary risk factors [5].

Gene polymorphisms are point mutations or allelic variations in the DNA, including single nucleotide polymorphisms [6]. A gene may be polymorphic if it occupies more than one allele gene locus in a population. Most of the studies on gene polymorphisms have been performed in cancer little is understood

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about the genetic components that may contribute to the development of COPD [7].

Smoke exposure causes harmful vascular effects by increasing the expression of vasoconstrictor and mitogenic factors such as endothelin-1 (ET-1) [8]. The autocrine effects (proinflammatory, vasoconstrictor, and mitogenic) of ET-1 may increase disease severity by being involved at the early stage of smoking-induced lung remodeling [8, 9].

In addition to many pathogenic mechanisms in inflammatory cycle cases, there being high ratio of Endotelin-1 (ET-1) in BAL liquids of COPD give rise to thought of this may have an important role in pathogenesis of COPD in the latest studies [10,11]. ET-1 gene, composed of five short exons and four introns, is on the p branch of the 6. chromosome. ET-1 gene codes preproendotelin which is a primary molecule and later on turns into amino acid ET-1 peptide [12,13]. When compared to other organs, ET-1 production and activity is on the highest level in the lungs. Because primary or active ET-1 peptide is not stored in the cell, this level is kept up by the activation of gene transcription. ET-1 take charge in locally effective ET-A and ET-B receptors in the lungs by producing autocrine-paracrine signal [12, 13]. The single nucleoid gen polymorphism in ET-1 is associated with ET-1 level [8, 14]. The single nucleoid gene polymorphism created by adding one adenine (+134 insA/delA), is seen as low as 138 bp in the transcription start region at 5'UTR of exon 1. Transinfection studies show that rather than translation effect of this polymorphism, it is responsible for the high ET-1 level provided by increased mRNA stability [12, 14]. And this is provided by unengaged energy affecting transcriptional stability by changing its second structure and amount in the stem loop, by creating different stem loops at 5'UTR transcripts of preproET-1 mRNA and adding adenine [14]. In a study, it is showed that 3A4A and 4A4A genotypes where +134 insA/delA polymorphism is, increases the risk of COPD [12]. COPD takes place with the interaction of genetic and environmental factors and ET-1 polymorphism which is about genetic load is worthy of attention [10, 11]. It is possible that ET-1 polymorphisms modulate the risk of developing COPD because of their effect on the maintenance of inflammation in the lungs of COPD patients and has not been adequately studied in the literature.

The aim of the present study was to research on the relation of COPD with single nucleotide gene polymorphism at ET-1 gene in a Turkish population.

2. Material and Methods

The study protocol was reviewed and approved by the Medipol Universty ethics committee. All patients signed a consent form. The study conforms to the relevant ethical guidelines for human and animal research.

2.1. Subjects

For the study 176 people, 87 of which has COPD and 89 of which is control group, were obtained. All participants for the study resided in Türkiye. To the study, people above 40 with at least 20 p/y smoking history were obtained.

Of the both patient group and control group anamnesis was taken and physical examinations were carried out. Epidemiologic characteristics like age, sex, kilo, height and smoking of patients and control group were recorded. After the assessment of spirometry results and cases, people with additional diseases, people who do not abide by the criteria of acceptance to the study, and people who has the exclusion criteria are not accepted for the research. Approximately 10 cc peripheral venous blood was taken from the participants who provide acceptance criteria and who signed informed consent form; these blood kept in hemogram tube with EDTA and at +4 degrees was studied on the condition of protocol transfer in Genetic Study and Diagnosis Laboratory in the rest of the day.

Study group: People whose FEV1/FVC ratio is below 70% at spirometry, males and females above 40 age, who has smoking history at least 20 p/y, who read and sign informed consent document. Control group: Male and females above 40 age, who has at least 20 p/y smoking history, whose FEV1/FVC ratio is above 70% at spirometry, who read and sign informed consent document. The patient who has lung diseases creating dyspnea symptoms like asthma, bronchiectasis, tuberculosis, sarcoidosis, interstitial lung disease (with x-ray, spirometry and history), comorbidities (malignancy, diabetes, congestive heart failure, liver and renal diseases) and the cases who did not sign informed consent document were excluded from the study.

During the pulmonary function tests, all tests were performed by the same investigator and a pulmonologist was the observer. All procedures were carried out according to the guidelines of the American Thoracic Society and the European Respiratory Society [15, 16].

2.2. Blood Collection and Genotyping

DNA Isolation

From the case participants constituting the study group, 1cc 0.5 M ethylenediaminetetraacetic acid (EDTA) (Sigma, ABD) and 9 cc blood sample was taken into tube. Isolated DNA was stored at +4 ºC.

Endothelin Gene -3A/-4A (-138 insertion/deletion) Polymorphism Scan. In this study, for endothelin gene -3A/-4A (-138 insertion/deletion) polymorphism analysis, PCR-RFLP method was used. To determine endothelin gene -3A/-4A (-138 insertion/deletion) the part containing the gene transformation was multiplied with the use of 5'GCTGCTTTTCTCCCCGTTAA3' and 5'CAAGCCACAAACAGCAGAGA3' primers

and PCR products of 195 bd were gained. Temperature conditions in PCR; at 95 °C for 5 mins denaturation, as 35 cycle at 95 °C for 1 min denaturation, at 58 °C for 1 min hybridization, at 72 °C elongation and at 72 °C for 7 mins last elongation was carried out (Biometra, USA). After PCR, the products were checked by putting 5µl into agarose gel of 2% on agarose gel electrophoresis; if seen any amplification of correct gene region, cutting operation was carried out with restriction endonuclease.

Cutting PCR Products with Restriction Endonuclease. So as to scan endothelin gene -3A/-4A (-138 insertion/deletion) polymorphism, BsiYI (Fermentas, Lithuania) enzyme was used. PCR products, in volume of 12.5 µl, were treated with 33mM Tris-acetate, 10mM Magnesium acetate, 66 mM Potassium acetate and RE buffer, including 0.1 mg/ml BSA (37 ºC, pH:7.9) and for each individual buffer-enzyme mixture of 10units/µl BsiYI. PCR product-enzyme-buffer mixture was left for rest at 55 ºC which is optimum working temperature of the enzyme for 14-16 hours for incubation.

Electrophoresis of Agarose Gel Restriction enzyme cut results were evaluated in agarose gel of 3%. Products which were cut by BsiYI enzyme were loaded on the gel by treating Bromene-phenol blue (Merck, Germany). It was carried out with 90-100V current for 30-50 mins (Biogen, USA). It was investigated under ultraviolet light (Spectroline, USA).

When the examples, which were carried out in agarose gel of 3%, were evaluated under ultraviolet light; individuals with -4A/4A genotype were not cut by enzyme and 195 base pair band was observed. Individuals with -3A/3A there was 195,176,19 base double pairs, individuals with -3A/3A there was 176,19 base pair bands observed.

2.3. Statistical Analysis

Data was evaluated with the help of SPSS v.26 (SPSS Inc., ABD) program. Allele ratio and genotype distribution, distribution amongst three genotype (3A3A, 3A4A, 4A4A) in the COPD patient and control group was analyzed by chi-square test. P value of comparison numbers including two alleles and two separate locus was confirmed with the help of Bonferroni method (Pc). Fisher exact test was applied to compare the small groups below the expected value of 5. Hardy-Weinberg equilibrium test was carried out with chi-square test. pulmonary function data analysis was carried out with t test. $p<0.05$ was accepted as statistical significance.

3. Results

Demographical characteristics of 176 subjects included in the study were summarized (Table 1). While the average age of COPD group was determined to be 60.4±8.9, the average age of control group was determined to be 50.2±8.3. When compared to control group, the average age of COPD group was significantly higher in the ($p<0.001$), (Table 1).

Table 1: Demographic characteristics of the subjects involved in the study.

	COPD patients (n:87)	Control group (n:89)	*P value*
Age; on average±SD	60.4±8.9	50.2±8.3	<0.001
Cigarette box/year	46.4±18.6	32.6±13	<0.001
Smoker/exsmoker	56/31	62/27	NS
BMI	24±4	27±7	0,06
Male/female	80/7	73/16	NS
FEV1(% predicted)	44.3±18	89±15	<0.001
FEV1/FVC (% predicted)	56±8	81±5	<0.001

BMI: Body mass index, NS: Not statistically significant, SD: Standard deviation.

Table 2: Endothelin 1 +134 insA/delA gene allele and genotype frequency of subjects.

Polymorphism	COPD patients (n= 87)	Control group (n=89)	P value (X^2)	Odss ratio	(95% CI)
134insA/delA					
Genotype					
3A3A	45 (51%)	63 (71%)		1,52	1.08-2.1
3A4A	41 (47%)	23 (2%5)	0.013 (6.7)	0.67	0.5-0.9
4A4A	1 (2%)	3 (4%)			
Allele frequency					
3A	131 (76%)	149 (84%)	0.044 (3.8)	0.7	0.62-0.98
4A	43 (24%)	29 (16%)			

CI: confidence interval, Odss ratio: risk ratio.

Whereas smoking amount in COPD group as box/year was 46.4±18.6 box/year on average, smoking amount in control group was 32.6±13 box/year. Smoking duration of COPD group was significantly higher than control group ($p<0.001$).

Genotype distribution and allele frequency is shown on table 2 for Endotelin-1 gene polymorphism (+134 insA/delA). As a result of statistical analysis, it is determined that +134insA/delA single nucleoid gene polymorphism is significantly higher in the patients than control group ($p<0.013$). There was no significant relationship between the respiratory function parameters, demographic characteristics and 134 insA/delA gene polymorphisms of the subjects (Table 3).

Table 3: The relation between subjects' respiratory function parameters, demographic characteristics and 134 insA/delA gene polymorphism.

	3A3A (n:45)	3A4A (n: 41)	*P value*
Age on average ± SD	59.9±7.9	61±9.7	NS
Cigarette box/year	45±16	48±21	NS
Smoker/ex-smoker	28/17	27/14	NS
BMI	25±3	24±4	NS
Male/Female	41/4	38/3	NS
FEV1(% predicted)	44±16	57±8	NS
FEV1/FVC (% predicted)	55±8	81±5	NS

BMI: Body mass index, NS: Not statistically significant, SD: Standard deviation.

4. Discussion

In our study, we researched Endotelin-1 gene (+134insA/delA) polymorphism, which plays an important role in COPD pathogenesis and we detected that ET-1 gene (+134insA/delA) is significantly higher in COPD cases than control group in Turkey ($p<0.001$). Endothelium is the source of factors with vasodilator and vasoconstrictor activities. Endothelin family, which is a powerful vasoconstrictor substance, was isolated in aorta endothelial cells at first [12, 17]. Endothelin isoforms have 3 types which are endothelin-1 and two small peptide ET-2 and ET-3. Each of them are products of different genes ET-1 is principally synthesized and excreted by endothelial cells [17]. ET-1 affects vascular smooth muscle cells, cardiac myocytes, fibroblasts and mesangial cells in the renal glomerulus in a mitogenic way. Furthermore, it also shows several effects on central and peripheral nervous system, gastrointestinal system, liver, urinary tract, male and female reproductive systems, eyes, skeletal system and the skin [17].

ET-1 participates in pulmonary hypertension as a mediator of changes in pulmonary vasculature. Its overexpression in the lung suggests that it has an important role in the initiation and progression of pulmonary hypertension (PH). In a study carried out by Kwon and his friends, they found out the ET-1 level of COPDs with pulmonary hypertension was significantly higher than the level of COPDs without pulmonary hypertension [18]. Beneficial effects of chronical treatment were stated with the help of specific ET receptor antagonists at experimental PH [19, 20].

ET-1 is a strong inotropic agent of tracheal and bronchial smooth muscle in the inflammatory system. It is related to various inflammatory diseases including COPD. ET-1, which is produced by vascular endothelin, bronchial epithelia, monocytes and fibroblasts in the lungs, is an important regulator in inflammatory area. Without having any starting irritator in inflammatory system mucosa, it is shown that ET-1 plays role in COPD pathogenesis by causing inflammatory cycle [21]. Many reasons causing COPD attacks rise in phlegm and is related to ET-1 concentration [22]. All these data indicate that ET-1 can play role in COPD ethiopathogenesis.

ET-1 shows local effects by signaling autocrine-paracrine in ET-A and ET-B receptors. At the same time, lungs are chief organs as to eliminate ET-1 from the cycle. In the latest studies, it is stressed that single gene mutation (SNP) in ET-1 gene probably rises ET-1 level by increasing ET-1-mRNA stabilization [12]. Hence, genetic polymorphism carriers, as their ET-1 level which is produced as a response to environmental stimulators, may have higher risk of COPD development comparing to non-carriers.

It can be said that ET-1 gene polymorphisms play role in regulating pathogenic mechanisms in COPD development, keeping inflammation at COPD patients in mind [10,11]. In a study carried out by Sampsonas and his friends [12], they drew attention to biologically and clinically probable significant polymorphic regions of ET-1 gene. SNP, which goes with adenine insertion, is found to be related to COPD. In the same study, in addition to ET-1 +134insA/delA allele polymorphism, G198T allele polymorphism having a relation with increased COPD risk was stated. Kaparianos et al. found that the +138 3A/4A and G198T SNPs of the ET-1 gene are probably not only involved in the pathogenesis of COPD, but also modulate the phenotypic expression of this disease, namely emphysema and chronic bronchitis [9]. In our study, only ET-1 +134insA/delA allele polymorphism was inspected and was found related to increased COPD risk.

Autocrine effects of ET-1 (pro-inflammatory, vasoconstrictor, mitogenic) can contribute to early remodeling in lungs related to smoking, and this can be related to disease severity for COPD and pulmonary hypertension. In a study Carratu and his friends found ET-1 level to be higher in COPDs with pulmonary hypertension than control group and related this result with disease severity [23]. In this situation, correlation between disease severity and polymorphism is expected. In another study, it was shown that in COPD cases G198T polymorphism correlates with disease phase and spirometry which shows disease severity [12]. In study Kaparianos and his friends made on COPDs, they related ET-1 gene polymorphism with annual FEV1 decrease and concluded the evaluation that COPD development and severity may be linked to this reason [9].

Contrarily, no relation between other ET-1 gene (+134insA/delA) polymorphism and spirometry and disease phase was found. In our study, there is not any detected relation between ET-1 gene (+134insA/delA) polymorphism and parameters showing disease severity. Besides, due to not doing ECHO, we couldn't comment on the relation between ET-1 gene polymorphism and pulmonary hypertension.

The relation between SNP which goes with adenine insertion in ET-1 polymorphism at COPD and COPD pathogenesis was found [12]. Patients, who probably carrying at least one 4A allele, produce mRNA creating PreproET-1, and this leads to 50-UTR transcription, and changes the second structure and amount of stem-loop and adenine insertion free energy [12]. In order to enlighten the underlying mechanisms beneath these changes, further studies are needed. If need any speculation about this topic, polymorphism may be developing as a response to ET-1 level increasing after smoking and this continues the inflammation and at last develops COPD. In our study, it was thought that, for detecting ET-1 gene polymorphism in COPD patients higher than control group, this polymorphism forms a basis for COPD development by playing an important role in COPD pathogenesis.

5. Conclusions

Our study is the first in our country relating ET-1(+134insA/delA) single nucleotide gene polymorphism and COPD phenotype according to the latest data. In consequence of our study, we think ET-1(+134insA/delA) gene polymorphism can distinctively take part in COPD pathogenesis and thus it can be a beneficial marker for identifying people with increased COPD risk. We think, in Türkiye single nucleoid gene polymorphism (+134insA/delA) in ET-1 gene increases the sensitivity to COPD development.

Limitations of the Study

When it comes to limitations of our study, the age and sex distribution was not homogenous between patients and control group, age in COPD patients was higher and in both groups sex distribution was on behalf of males. As a result of this, we observed smoking and consequently COPD shows male predominance and the hesitance of females comparing to males to involve in the study.

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None.

Conflict of Interests

All authors declare there is no conflict of interest**.**

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Author Contributions

MD designed the research. GK and ECS participated in data collection and data analysis. MD and GK wrote the manuscript, GK and MB read and approved the final script.

Ethical Approval

No ethical approval was needed for this study.

Data sharing statement

Not applicable for this study.

Consent to participate

Consent was obtained from the patient and control groups participating in the study.

Informed Statement

All patients signed a consent form.

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