ACE gene I/D polymorphism and arterial hypertension in patients with COPD

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Abstract

Background: Cardiovascular diseases (CVDs) are common in people with chronic obstructive pulmonary disease (COPD), and their presence is associated with an increased risk for hospitalization, longer length of stay and all-cause and CVD-related mortality. We assessed the role of angiotensin-converting enzyme (ACE) gene polymorphism in the occurrence of arterial hypertension (AH) in patients with COPD.

Methods: The study group consisted of 96 patients. Group 1 had 25 individuals with COPD, Group 2 had 23 individuals with AH and Group 3 had 28 individuals with COPD and AH. The control group consisted of 20 healthy subjects. I/D genotypes of ACE were determined by polymerase chain reaction amplification.

Results: The frequency distribution of polymorphic genotypes of the gene encoding ACE and assessment of compliance with the Hardy-Weinberg population equilibrium were carried out in groups of patients with COPD, AH and COPD + AH. The frequencies of the genotype responsible for I/D polymorphism of the ACE gene in the control and experimental groups were not found to deviate significantly from the Hardy–Weinberg equilibrium. The results of the study have not demonstrated any significant impact of alleles of ACE genes or ACE genes on occurrence of diseases such as COPD, AH and combinations thereof. However, analysis of odds ratio has demonstrated that the presence of the D allele of the ACE gene may increase the risk for occurrence of the COPD + AH (OR = 1.26).

Conclusion: The data obtained in the study allow suggesting that the presence of D allele of the ACE gene may increase the risk for AH in patients with COPD.

Keywords

chronic obstructive pulmonary disease • arterial hypertension • angiotensin-converting enzyme gene, insertion-deletion polymorphism

Polimorfismul genei I/D a ACE si hipertensiunea arteriala la pacientii cu BPOC

Rezumat

Context general. Bolile cardiovasculare sunt frecvente in randul pacientilor cu boala pulmonara obstructiva cronica (BPOC), iar prezenta acestora se asociaza cu un risc crescut de spitalizare, cu durata mai mare de spitalizare si cu mortalitate crescuta de toate cauzele si de cauze cardiovasculare. In cadrul acestui studiu am evaluat rolul pe care il joaca polimorfismul genei de conversie a angiotensinei (ACE) in apariitia hipertensiunii arteriale (HTA) la pacientii cu BPOC.

Metoda. Grupul de studiu a fost reprezentat de 96 de pacienti: Grupul 1 (25 indivizi cu BPOC), Grupul 2 (23 indivizi cu HTA), Grupul 3 (28 indivizi cu BPOC si HTA). Grupul control a fost reprezentat de 20 de subiecti sanatosi. Genotipurile I/D ale ACE au fost determinate prin tehnica de amplificare genica in lant.

Rezultate. Au fost evaluate frecventa de distributie a genotipurilor polimorfe ale genei ce codifica ACE si compliancia cu ajutorul echilibrului Hardy-Weinberg in grupul pacientilor cu BPOC, HTA si combinatia BPOC + HTA. Frecventa cu care a survenit genotipul responsabil de polimorfismul I/D al genei ACE in grupul control si in grupurile experimentale nu a diferit semnificativ de echilibrul Hardy-Weinberg. Rezultatele studiului nu au demonstrat nici un impact semnificativ al alelelor sau genelor ACE asupra aparitiei unor afectiuni ca BPOC, HTA sau combinatia acestora. Oriocum, analiza riscului exprimat ca odds ratio a demonstrat ca prezenta alelei D a genei ACE poate creste riscul de aparitie de BPOC + HTA (OR = 1.26).

Concluzii. Datele obtinute in acest studiu sugereaza ca prezenta alelei D a genei ACE poate sa creasca riscul de HTA la pacientii cu BPOC.

Cuvinte-cheie

boala pulmonara obstructiva cronica • hipertensiune arteriala • gena enzimei de conversie a angiotensinei • polimorfism insertie-deletie

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Introduction

Chronic obstructive pulmonary disease (COPD) is a common respiratory health problem characterized by persistent respiratory symptoms, such as dyspnoea, cough and airflow limitations (1). COPD represents one of the leading causes of death worldwide. According to the World Health Organization (WHO), COPD retains the III, IV and V places in upper-middle-, lower-middle- and high-income countries respectively, and by 2020 will attain the third place all over the world (2).

The frequency of comorbidities in COPD strongly varies between studies, given the heterogeneity of the definitions used, the study populations, patient COPD severity and age (3). Despite these differences, most studies conclude to a high prevalence of COPD patients with associated comorbidities, from 70% to virtually all patients (4). Cardiovascular diseases (CVDs) are arguably the most important comorbidities in COPD. CVDs are common in people with COPD, and their presence is associated with an increased risk for hospitalization, longer length of stay and all-cause and CVD-related mortality (5). The mechanisms that underlie the association between COPD and CVDs are not well understood, but several processes are thought to be important and may interact with each other. These include lung hyperinflation, hypoxaemia, pulmonary hypertension, systemic inflammation and oxidative stress, exacerbations, shared risk factors and shared genetics, as well as COPD phenotype (6).

Endothelial dysfunction fundamentally contributes to the development of atherosclerosis that finally leads to coronary heart disease (CHD). The process is further accelerated by systemic inflammation and oxidative stress (7). As a result, approximately one out of six COPD patients suffers from concomitant CHD (8). Endothelial function is known to be impaired in subjects with chronic heart failure (CHF), which is a syndrome rather than a disease (9). Left heart failure was diagnosed first time in one out of five COPD patients after extensive cardiovascular work-up (10). Vice versa, one out of three heart failure patients suffers from obstructive ventilation disorders (11). Arterial hypertension (AH) is comparably frequent in COPD patients and the general population. The prevalence of AH is about 50% and increases with age (12). Nevertheless, the overall elevated cardiovascular risk in COPD may be a product of this common condition and potentiating effects of other risk factors such as diabetes mellitus and cigarette smoking. Moreover, higher central blood pressure values and arterial stiffness are also found indicating premature atherosclerosis (8).

The coexistence of COPD, CVDs and major risk factors for CVD highlights the crucial need for the development of strategies to screen for and reduce cardiovascular risks associated with COPD. Both AH and COPD are genetically determined conditions with multiple genes, combinations of genes, inter-gene interactions and epigenetic processes responsible for their occurrence. When adverse genetic and external factors combine, a disease is formed (13). The relationship between the polymorphism of the gene encoding angiotensin-converting enzyme (ACE) and COPD has been addressed in numerous prior studies; however, these studies have yielded ambivalent results (14–16). Prior studies have demonstrated the role of genetic factors in susceptibility to COPD; in part, susceptibility to COPD was found to be associated with polymorphism of proteinase-activated receptor-1 (17), plasminogen activator inhibitor-1 (18) and β2-adrenergic receptors (19). A study of relationship between COPD and ACE activity has shown the activity of ACE to depend on oxygen concentrations in the blood; therefore, an increase in ACE levels due to COPD-associated hypoxia may cause severe tissue damage (20).

Thus, the aim of this study was to establish the role of ACE gene polymorphism in the occurrence of AH in patients with COPD.

Methods

The study group consisted of 96 patients admitted to the Ternopil University Hospital. We stratified patients into three groups: Group 1 (25 patients with COPD), Group 2 (23 patients with AH) and Group 3 (28 patients with COPD + AH). The control group consisted of 20 healthy subjects.

Inclusion criteria

The inclusion criteria were male patients 40 to 60 years of age at screening with a diagnosis of COPD and/or AH and informed consent form signed by patients prior to their participation in any study-related procedures. COPD was diagnosed according to Order 555 of MoH of Ukraine dated 27 June 2013 and according to the guidelines published by the American Thoracic Society and European Respiratory Society (GOLD, 2013). Airway obstruction was assessed using GOLD classification, 2008. The diagnosis of COPD with moderate (Stage 2) airway obstruction was confirmed with compatible clinical features concurrent with airflow limitation defined as forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) ratio less than 0.70 (FEV1/FVC ratio of 50–79% predicted).

The diagnosis of AH (Stage I) was made according to 2018 ESC/ESH Guidelines for the management of AH (21). Systolic (140–159 mmHg) blood pressure and/or diastolic (90–99 mmHg) blood pressure were considered as the
presence of Stage I AH. Left ventricular hypertrophy was confirmed by an electrocardiogram.

Exclusion criteria
Patients with bronchial asthma; α1-antitrypsin deficiency; active tuberculosis; lung cancer; significant bronchiectasis; sarcoidosis; pulmonary fibrosis; interstitial lung disease; signs and symptoms of clinically significant neurological, psychiatric, renal, hepatic, immunological, gastrointestinal and urogenital disorders; musculoskeletal conditions; disorders of the skin and sensory organs; endocrine disorders (uncontrolled diabetes or thyroid disease) or uncontrolled haematological disease; unstable liver disease; unstable or life-threatening heart disease; cancer not completely disease free for a minimum of 5 years and any drug, substance or alcohol abuse were excluded.

Sampling of venous blood for genotyping was performed under sterile conditions into 2.7 mL Monovettes with potassium salt of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant; the samples were frozen and stored at -20°C. Molecular genetic studies were performed with extraction of DNA and with use of polymerase chain reaction (PCR) and further analysis for the length of restriction fragments. DNA was extracted from peripheral blood leukocytes using a standard salt precipitation method. Genotyping for the ACE I/D was performed using PCR-based restriction fragment length polymorphism (RFLP). The primers used were 5′-GATGCGCACAAGGTCCT-GTC-3′ (forward) and 5′-CAGGGTGCTGTCCAC-ACTGG ACCCC-3′ (reverse). The PCR products were digested with 3 U of Tth111I (Fermentas), and the fragments were separated on a 3% agarose gel containing ethidium bromide and visualized with UV light. To assess genotyping reliability, we performed double sampling RFLP–PCR in more than 20% of the samples and found no differences (22).

Statistical analysis
Statistical data analysis was carried out using Statistica 7.0 software. Assessment of genotypes of the selected sample for conformity to general population sample was guided by the Hardy–Weinberg principle. The observed frequencies and the expected frequencies calculated from the expression $p^2 + 2pq + q^2 = 1$ (Hardy–Weinberg equilibrium) were compared using Pearson chi-square, $\chi^2$. In case of probability value $p > 0.05$, a null hypothesis of equal samples was accepted, i.e. the selected sample was equivalent to the general population. Comparative analysis of frequency tables was performed using Pearson chi-square, $\chi^2$, and Fisher’s exact $p$, two tailed (in cases when expected frequencies of individual parameters did not exceed 5). To assess the impact of the factor (the presence of a certain genotype or an allele of a gene) on the occurrence of the disease, odds ratio (OR), its 95% confidence interval (CI) and probability value $p$ were calculated.

Results
The frequency distribution of polymorphic genotypes of the gene encoding ACE and assessment of compliance with the Hardy–Weinberg population equilibrium were carried out in groups of patients with COPD, AH and COPD + AH combination. The frequencies of the genotype responsible for I/D polymorphism of the ACE gene in the control and experimental groups were not found to deviate significantly from the Hardy–Weinberg equilibrium ($p > 0.05$; Table 1). The respective frequencies for the genotypes of the ACE gene were as follows: 28.0% for I/I, 56.0% for I/D and 16.0% for D/D in Group 1 with COPD; 30.4% for I/I, 52.2% for I/D and 17.4% for D/D in Group 2 with AH; 32.1% for I/I, 42.9% for I/D and 25.0% for D/D in Group 3 with COPD + AH and 25.0% for I/I, 60.0% for I/D and 15.0% for D/D in the control group (Table 2).

The frequencies of alleles for the ACE gene in patients with COPD, AH and COPD + AH and in control group patients are given in Table 3. In the COPD group, the established distribution was 56.0% for ACE I allele and 44.0% for ACE D allele; in the AH group, 56.5% and 43.5% and in the COPD + AH group, 53.6% and 46.4%. However, these data significantly did not differ from the control group.

### Table 1. Hardy–Weinberg equilibrium of the ACE gene I/D polymorphism in COPD, AH and their combination

| Genotype         | COPD  |        | AH    |        | COPD + AH |        | Control |        |
|------------------|-------|--------|-------|--------|-----------|--------|---------|--------|
|                   | Expected | Observed | Expected | Observed | Expected | Observed | Expected | Observed |
| Common homozygotes |       |       |       |       |           |        |         |        |
| I/I               | 7.8    | 7      | 7.3   | 7      | 8         | 9      | 6.1     | 5      |
| Heterozygotes     | 14.6   | 14     | 11.3  | 12     | 13.9      | 12     | 9.9     | 12     |
| Rare homozygotes  | 2.6    | 4      | 4.4   | 4      | 6.1       | 7      | 4       | 3      |

Chi-square, $\chi^2$:

- $\chi^2 = 0.86, df = 2, p > 0.05$
- $\chi^2 = 0.09, df = 2, p > 0.05$
- $\chi^2 = 0.52, df = 2, p > 0.05$
- $\chi^2 = 0.89, df = 2, p > 0.05$

ACE, angiotensin-converting enzyme; COPD, chronic obstructive pulmonary disease; AH, arterial hypertension.
Table 2. Genotype frequencies of the ACE gene I/D polymorphism in COPD, AH and their combination

| Genotype frequencies | COPD  | AH   | COPD + AH | Control |
|---------------------|-------|------|-----------|---------|
|                     | n     | %    | n         | %       |
| I/I                 | 7     | 28.0 | 7         | 30.4    |
| I/D                 | 14    | 56.0 | 12        | 52.2    |
| D/D                 | 4     | 16.0 | 4         | 17.4    |

Fisher’s exact $p$, two tailed (disease/control group) $p = 1.0$ $p = 0.75$ $p = 0.75$ –

ACE, angiotensin-converting enzyme; COPD, chronic obstructive pulmonary disease; AH, arterial hypertension.

Table 3. Allele frequencies of the ACE gene I/D polymorphism

| Allele frequency | COPD  | AH   | COPD + AH | Control |
|-----------------|-------|------|-----------|---------|
|                 | n     | %    | n         | %       |
| ACE I allele    | 28    | 56.0 | 26        | 56.5    |
| ACE D allele    | 22    | 44.0 | 20        | 43.5    |

Pearson’s chi-square, $\chi^2$ (disease/control group) $\chi^2 = 0.01$, df = 1, $p = 0.92$ $\chi^2 = 0.02$, df = 1, $p = 0.89$ $\chi^2 = 0.02$, df = 1, $p = 0.89$ –

ACE, angiotensin-converting enzyme; COPD, chronic obstructive pulmonary disease; AH, arterial hypertension.

Table 4. OR for alleles in different study groups

| Group      | Allele I allele | Allele D allele |
|------------|----------------|----------------|
|            | OR     | 95% CI | $p$  | OR     | 95% CI | $p$  |
| COPD       | 1.04   | 0.45–2.40 | >0.05 | 0.96   | 0.42–2.22 | >0.05 |
| AH         | 1.06   | 0.45–2.50 | >0.05 | 0.94   | 0.40–2.21 | >0.05 |
| COPD + AH  | 0.94   | 0.42–2.13 | >0.05 | 1.26   | 0.47–2.39 | >0.05 |

OR, odds ratio; ACE, angiotensin-converting enzyme; CI, confidence interval; COPD, chronic obstructive pulmonary disease; AH, arterial hypertension.

Table 5. OR for genotypes in different study groups

| Group      | Genotype | I/I | I/D | D/D |
|------------|----------|-----|-----|-----|
|            | OR       | 95% CI | OR | 95% CI | OR | 95% CI |
| COPD       | 1.17     | 0.31–4.44 | 0.85 | 0.26–2.80 | 1.08 | 0.21–5.50 |
| AH         | 1.31     | 0.34–5.05 | 0.73 | 0.22–2.44 | 1.19 | 0.23–6.11 |
| COPD + AH  | 1.42     | 0.39–5.14 | 0.50 | 0.16–1.61 | 1.89 | 0.42–8.43 |

OR, odds ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease; AH, arterial hypertension. $p$ value >0.05 in all cases.

The results of the study given in Table 4 have demonstrated absence of a statistically significant relationship between the factor (presence of I or D alleles) and occurrence of the disease ($p > 0.05$). The results of the study presented in Table 5 have also demonstrated absence of a statistically significant relationship between the factor (presence of I/I, I/D or D/D genotypes) and occurrence of the disease ($p > 0.05$). However, analysis of OR has demonstrated that the presence of the D allele and D/D genotype of the ACE gene may be associated with a higher risk of the AH occurrence in COPD patients.

Analysis of dominant and recessive types of inheritance for the ACE gene (alleles I and D) did not establish any significant differences in the groups of COPD, AH, and their combination ($p > 0.05$).

Discussion

ACE is an endopeptidase consisting of two catalytic domains; this enzyme is usually expressed by endothelial, epithelial and neuronal cells (23). It exists both in a membrane-bound form (ACE) and in a soluble form (sACE), the latter produced through exposure to zinc metalloprotease (referred to as “ACE secretase”), which cleaves the mature membrane-bound ACE in the juxtamembrane extracellular domain to release the large extracellular part of the enzyme (23, 24). The known function of ACE is associated with the renin–angiotensin system, wherein ACE catalyzes the synthesis of angiotensin II vasoconstrictor from its non-vasoactive precursor, angiotensin I, and is also responsible for inactivation of vasodilator bradykinin (25). Association between I/D polymorphism of the ACE gene and the risk for COPD is a very timely and widely studied issue; however, existing data need to be clarified and appended (15, 26, 27). The low activity of ACE was recognized to play a positive role in the development of COPD (28). It is worth noting that the DD genotype of ACE is generally associated with increased circulating and cellular levels of ACE and increased cardiovascular risk (29). However, most of overall ACE activity in the body is located in the pulmonary tissue,
and its activity is additionally increased in chronic hypoxia, which develops in COPD. Therefore, ACE in the pulmonary tissue may be involved in the pathogenesis of pulmonary hypertension secondary to COPD. Kanazawa et al. show insertion/deletion (I/D) polymorphism in the ACE gene to be related to increases in plasma ACE levels (30). Analysis of other published studies on whether ACE gene insertion/deletion polymorphism was associated with the risk of COPD suggests lack of significant association between I/D polymorphism and COPD. Carriers of II and ID genotypes had significantly lower levels of circulating ACE than subjects with DD genotype (31). A noteworthy detail is that an interaction between allele 894G of the gene encoding endothelial nitrogen oxide synthase and allele I of the ACE gene may reduce vasoconstriction and increase vasodilatation (32), which is a positive effect in both COPD and hypertension. In our study, greater proportions of II homozygotes were seen among both patients with AH and patients with combined COPD + AH; however, no significant relationship was found between I allele and the incidence of conditions explored in this study. In the meantime, II genotype was associated with lower respiratory rates, which may be an indirect effect of vasodilatation.

The probable association between the D allele and D/D genotype of the ACE gene with the incidences of COPD + AH combination presented in results is attributable to the fact that, along with the altered endothelial responses, the D-allele of the ACE gene increases capillary permeability, ACE production, activation of angiotensin II, and also degradation of bradykinin (33). Given that the higher plasma levels of ACE depend on the DD genotype, an assumption can be made that the I allele may have a protective role against comorbidities in patients with COPD, as dependent on higher plasma ACE levels.

The present study has some limitations that should be considered in the interpretation of our results: sample size is too small, that is why it is difficult to find significant relationships from the data; the inclusion in the study group only of patients with combined COPD + AH; however, no significant relationship was found between I allele and the incidence of conditions explored in this study. In the meantime, II genotype was associated with lower respiratory rates, which may be an indirect effect of vasodilatation.

We did not observe a significant correlation between I/D polymorphism in the ACE gene and the risk of AH in COPD patients; therefore, comprehensive studies on Ukrainian patients are required. In addition, the trend towards increased D/D genotype frequency in patients with COPD and AH (although not significantly increased) suggests that this genotype may be associated with a higher risk of AH occurrence in COPD patients.

**Ethical approval**

The study protocol was approved by the Medical Ethics Committees of I. Horbachevsky Ternopil National Medical University (No 47-25/02/2017), and the study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 1983. Informed consent was obtained from all patients.

**Competing interests**

The authors declare that they have no competing interests.

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