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DD genotype of the I/D Angiotensin-Converting Enzyme Gene Polymorphism is a higher Risk for Atopic Asthma

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ABSTRACT Background: Atopic asthma begins with exposure to allergens causing airway hyperresponsiveness and chronic inflammation. Angiotensin II involved in the pathogenesis of atopic asthma by causing airway hyperresponsiveness. Angiotensin II levels are influenced by the levels of ACE and ACE levels are influenced by I / D ACE gene polymorphisms. Study about the role of I/D ACE gene polymorphism in the incidence and severity of asthma in several countries and ethnic showed inconsistency result. Study in Indonesian subjects had never been reported. **Method:** This Case-control study aimed to evaluate the influence of I/D ACE gene polymorphism in the incidence and severity of atopic asthma. Eighty subjects aged 15-50 years including Forty non–asthmatic and Forty atopic asthmatic patients were included in this study. **Result:** DD genotype with atopic asthma (85.7%) was higher than non-atopic asthma (14,3%). Non-DD genotype (ID, II) with atopic asthma (46.6%) was lower than atopic asthma (53.4%). DD genotype of the ACE gene had a risk 6.8 times to become atopic asthma than non-DD genotype (OR=6.8,p=0.05). DD genotype of the ACE gene was not a risk factor for severe obstruction (OR=0.50, p=0.48) and uncontrolled atopic asthma compared to non-DD genotype (OR = 1.8,p=0.49). **Conclusion:** This study concluded that DD genotype of the ACE gene was a risk factor for the incidence of atopic asthma but not a risk factor for severe obstruction and uncontrolled atopic asthma.

KEYWORDS Polymorphism, ACE gene, atopic asthma, severe obstruction, uncontrolled asthma

Introduction

Asthma is 1 of 10 significant causes of morbidity & mortality in Indonesia. The Indonesian National Household Health Survey in 1995 reported asthma was 5th rank of 10 cause of morbidity as well as bronchitis & emphysema. Asthma prevalence in Indonesia around 5-7% and about 75% asthma is atopic asthma[1] Atopic asthma is a chronic airway disease. Its major characteristics include inflammation, bronchial hyperresponsiveness, obstruction and airway remodelling [2]. It is widely accepted that atopic asthma is a complex disease, and both environmental factors (allergens, viruses, and occupational exposures) and genetic factors contribute to its inception and evolution [2]. Angiotensin-converting enzyme, a key enzyme of the renin-angiotensin system, is mainly expressed in the lung and plays an essential role in the pathogenesis of asthma [3].

The angiotensin-converting-enzyme converts angiotensin I to angiotensin II, which may be involved in the aetiology of asthma by interaction with bronchial muscle and cause bronchial hyperresponsiveness [4]. The human ACE gene is located on chromosome 17q23, where an insertion/deletion (I/D) polymorphism in intron 16 has been identified. This polymorphism is based on the insertion (I) or deletion (D) of a 287-bp nonsense DNA fragment. It affects the serum ACE level and has been investigated as a potential susceptibility factor for asthma [5]. A large number of studies have reported the association between the I/D

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Table 1 Characteristics of the study group.

Phenotype	Atopic asthma n = 40	Control n = 40	P
Age (mean±SD)	32,1 (7,7)	29,1 (7,4)	p=0,081
Sex (F/M)	20/20	20/20	

*Independent t-test

Table 2 ACE I/D genotype distributions in the study group.

Genotype I/D genACE	Atopic Asthma	Control	p*	OR	CI
DD	6 (85.7%)	1 (14.3%)	0.05	6.8	0,78 – 60
Non-DD (DI + II)	34 (46.6%)	39 (53.4%)			

* Chi-square test

polymorphism of the ACE gene and the risk of asthma [6-12], but the results were inconsistent. There was no study have been reported the frequency of ACE gene polymorphisms in atopic asthmatic in Indonesian subjects. It is also not known whether the presence of these genotypes is a risk factor for atopic asthma severity. We aimed to investigate the frequency of ACE gene polymorphisms in atopic asthmatic patients and to determine the correlation between ACE I/D gene polymorphisms to severe airway obstruction and uncontrolled atopic asthma.

Materials and Method

Characteristics of the patients

Eighty Indonesian subjects aged 15-55 years, including 40 atopic asthmatic patients and 40 non-asthmatic control subjects, participated in this study. This study was performed in June-September 2014. Atopic asthmatic subjects were recruited from out-patient clinics of Hasanuddin University Hospital, Department of Internal Medicine in Makassar Indonesia. Atopic asthma was diagnosed by a history of intermittent wheezing and the presence of reversible airway obstruction as defined by at least a 12% improvement in forced expiratory volume in 1s (FEV1) following bronchodilator test. Asthmatic subjects underwent skin prict test (SPTs) and were classified as atopic asthma if the result was positive. The inclusion criteria for atopic asthmatic subjects were not in acute exacerbation status and non-smoker. Atopic asthmatic subjects were classified into uncontrolled and controlled atopic asthma using Asthma control test. There was 15 subject with uncontrolled atopic asthma and 25 with controlled asthma. The degree of obstruction was defined by using spirometry and included severe obstruction if FEV1% > 50%.

Ethics

The Ethics committee had approved this study of Medical Faculty Hasanuddin University with number; 01186/H.4.8.4.5.31/PP.36-KOMETIK/2014.

Genotyping

Genomic DNA was isolated from blood lymphocytes using the phenol-chloroform method, as previously described. Polymerase chain reaction (PCR) amplification of the ACE gene I/D polymorphisms were performed in a final volume of 50-µL containing 100 ng DNA, ten pmoL each of the primers (forward 5_CTGGAGACCACTCCCATCCTTTCT3_ and reverse 5_GATGTGGCCATCACATTCGTCAGA3_), 0.2 mmol/L of each of the dNTPs, 1.5 mM MgCl2, 1U Taq polymerase, and 10X reaction buffer (Tris-HCL, 0.5% nondiet P40, 0.5% between 20 and 50% glycerol, pH 8.0) (MBI Fermantas, Lithuania). PCR cycling conditions were 94°C for 5 minutes, annealing at 58°C for 1 minute, extension at 72°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 58°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR products were separated on a 2% agarose gel, stained with ethidium bromide, and visualized with UV transillumination. The samples with the D/D genotype were amplified (as described above)with the following primers to prove genotype: forward primer 5_TGGGACCACAGCGCCCGCCACTAC3_ and reverse 5_TCGCCAGCCCTCCCATGCCCATAA3_.[13]

Statistics

Data were analysed with SPSS software (Scientific Package for Social Sciences, Version 17.0, SPSS, Inc., Chicago, IL, USA). The Chi-square was performed to test for comparison of differences in genotype. Odds ratio and 95% confidence interval values were calculated for the demonstration of an association between atopic asthma and ACE I/D polymorphisms. A value of p = 0.05 or less was considered to be significant.

Result

The characteristics of atopic asthmatics and the control group are shown in Table 1. There were no differences between groups using age and gender. The ACE genotype distributions in the study groups are shown in Table 2. The frequencies of genotypes were significantly different in atopic asthmatics and controls. DD genotype with atopic asthma (85.7%) was higher than non-atopic asthma (14,3%). Non-DD genotype (ID, II) with atopic asthma (46.6%) was lower than atopic asthma (53.4%). DD genotype of the ACE gene had a risk 6.8 times to become atopic asthma than non - DD genotype (p = 0.05).

I/D genotype of ACE gene distributions according to severe obstruction are shown in Table 3. There was no significant difference between DD and non-DD.

I/D genotype of ACE gene distributions according to the degree of asthma control are shown in Table 4. There was no significant difference between DD and non-DD.

Table 3 ACE I/D genotype distributions according to the degree of obstruction.

Genotype I/D genACE	Severe obstruction	Non-severe obstruction	p*	OR	CI
DD	1 (16.7%)	5 (83.3%)	0.48	0.5	0,05-4,83
Non-DD (DI + II)	10 (28.6%)	24 (71.4%)			

* Chi-square test

Table 4 ACE I/D genotype distributions according to the degree of asthma control.

Genotype I/D genACE	Severe obstruction	Non-severe obstruction	p*	OR	CI
DD	3 (50%)	3 (50%)	0.49	1.8	0,3 – 10,3
Non-DD (DI + II)	12 (35,3%)	22 (64,7%)			

* Chi-square test

Discussion

Asthma is a polygenic disorder and also represent genetic heterogeneity. ACE gene is potential candidate genes supposed to be involved in the pathogenesis of atopic asthma. In this study, we found a significantly higher frequency of the ACE gene DD mutation among atopic asthmatic patients, compared to healthy control subjects. DD genotype of the ACE gene had a risk 6.8 times to become atopic asthma than non-DD genotype. Our findings are mainly consistent with the previous studies. Similar results were reported by Lee et al. [14] and Nakahama et al. [15]. Benessiano et al. reported a higher prevalence of the ACE DD genotype in 79 asthmatic patients compared with healthy subjects and patients with non-asthmatic. [7] Similarly, Gao et al. found a higher prevalence of DD genotype of the ACE gene in patients with bronchial hyperresponsiveness. They also observed higher FEV1 and FEV1/FVC values in asthmatics carrying non-DD-alleles[16]. Conversely, other studies have found no association between the ACE gene polymorphisms and asthma. Bora et al. reported the ACE gene I/D polymorphisms did not play role in the development of asthma in Turkish subject. [17] Tomita et al. found a similar distribution of genotype and serum ACE levels between healthy control subjects and asthmatic patients [18]. Chagani et al. reported no association between the ACE gen and asthma development and also the severity of asthma[6]. Similarly, Nakahama et al. could find no association between ACE gene polymorphisms and an increased risk for asthma or asthma severity [19]. A recent meta-analysis suggested that I/D polymorphism of the ACE gene would be a risk factor for asthma. Results of this meta-analysis indicated that DD genotype of ACE increased risk of asthma and this risk is more evident in Asians represent by China, Japan and South Korean subjects. [20]

However, in this study, there was no association of asthma severity with the I/D gene ACE polymorphisms. Previous studies have shown that the ACE gene DD mutation may contribute to the pathogenesis of asthma by affecting serum ACE levels. ACE gene insertion/deletion mutations influence serum ACE levels and DD genotype have higher ACE level than non-DD. [21] Higher levels of ACE will increase angiotensin I conversion to angiotensin II. Angiotensin II, which has mitogenic effects and enhances contractility of the smooth muscle and also cause bronchial hyperresponsiveness. [4] Conversely, ACE might manifest favourable effects in asthma by inactivating bradykinin. Production of bradykinin causes oedema and contraction of the smooth muscle in the airways or increased release of leukotrienes and histamine and a worsening of symptoms. [22] Higher ACE will cause a decreased level of bradykinin.

Our findings are mainly consistent with the previous study. Benessiano et al. showed an increased frequency of the ACE DD genotypes among asthmatic patients, but no correlation could be demonstrated between disease severity and the increased frequency of this genotype [7]. Eryuksel et al. found a higher frequency of the ACE DD gene mutation in Turkish asthmatic patients compared with non-asthmatics, suggesting that this ACE gene polymorphism may be a risk factor for asthma but does not increase the severity of the disease. [12]

There were several limitations in our study that should be considered. Firstly, we did not evaluate serum ACE levels. Secondly, our population may not be representative of the Indonesian population as a whole. Sample collected from Makassar only from one region of Indonesian. Different ethnic origins and from all parts of Indonesian may be more representative. This study concluded that DD genotype of the ACE gene was a risk factor for the incidence of atopic asthma but was not a risk factor for severe obstruction and uncontrolled asthma.

Conflict of interest

The authors have no conflicts to disclose. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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