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ORIGINAL ARTICLE

# *Alu* insertion/deletion of *ACE* gene polymorphism might not affect significantly the serum bradykinin level in hypertensive patients taking ACE inhibitors

**Widodo [a,1,*], Shila Wisnasari [b,d,1], Mohammad Saifur Rohman [c], Lowry Yunita [c,g], Mifetika Lukitasari [d], Maulidiyatun Nuril [e], Kholifah Holil [e], Dian Laila Purwaningroom [f]**

[a] *Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia*
[b] *Biomedical Science, Faculty of Medicine, Brawijaya University, Malang, Indonesia*
[c] *Department of Cardiology and Vascular Medicine, Faculty of Medicine, Brawijaya University and Saiful Anwar General Hospital, Malang, Indonesia*
[d] *Nursing Science, Faculty of Medicine, Brawijaya University, Malang, Indonesia*
[e] *Biology Department, Faculty of Science and Technology, Islamic State University of Maulana Malik Ibrahim, Malang, Indonesia*
[f] *Faculty of Health Science, Muhammadiyah University, Ponorogo, Indonesia*
[g] *Department of Cardiology and Vascular Medicine, RSUD Prof. Dr. W. Z. Johanes, Kupang, Nusa Tenggara Timur, Indonesia*

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**Abstract** *Background:* Angiotensin I-converting enzyme (ACE) has two homologous catalytic domains, the N- and C-domains. Our previous study suggested that *Alu* insertion (I allele) in the intron 16 of ACE resulted in premature codon termination. The I allele has only one active site in the N-domain while the *Alu* deletion (D allele) still has two active sites of ACE. Therefore the effect of I/D polymorphism of ACE on the enzyme's ability to catalyse bradykinin is still not widely known.

*Aims:* This study aimed to examine the serum bradykinin level in hypertensive patients with I/D polymorphism of ACE, who were treated with ACE inhibitor.

*Subjects and methods:* The serum bradykinin and I/D polymorphism have been detected in 64 hypertensive patients taking ACE inhibitor (lisinopril or captopril) for at least eight weeks with good medication adherence. The binding affinity of ACE with its receptor was calculated by molecular docking.

*Results:* The findings show that genotype II is more frequent in the population the researchers observed (53.12%) compared to ID (23.44%) and DD (23.44%) variances. On the other hand, the bradykinin level is not affected by genotype of the ACE genes on the population. Bradykinin increases in

* Corresponding author at: Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Jl. Veteran, Malang, Indonesia.
E-mail address: widodo@ub.ac.id ( Widodo).
[1] Equally contributor.

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patients with genotype II who are given captopril, but decreases in patients treated with lisinopril. Nevertheless, there is no statistically significant difference.

*Conclusion:* This study suggests that the polymorphism might not significantly affect the serum bradykinin level in hypertensive patients taking ACE inhibitors.

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## 1. Introduction

Angiotensin I-converting enzyme (ACE) is a zinc metallopeptidase that plays an important role in blood pressure regulation [1–3]. The ACE has two homologous catalytic domains, the N- and C-domains, which are able to cleave angiotensin I and bradykinin [4,5,2]. The C-domain of ACE is more efficient in cleaving angiotensin I into vasopressor angiotensin II [4]. However, reports regarding the bradykinin binding site to the ACE are still limited. This information is necessary to measure the strength of binding competition between bradykinin and the angiotensin I to the ACE, although the ACE inhibitors generally have a higher affinity for the bradykinin than the angiotensin I binding sites [5]. This knowledge is essential for developing more specific ACE inhibitors on angiotensin I binding site as a hypertension drug that has no side effects on cough.

Our previous study suggested that *Alu* insertion (I) in the intron 16 of ACE resulted in premature codon termination, so the protein has only one active site in the N-domain while the *Alu* deletion allele (D) still has two active sites. The meta-analysis indicated that the D allele is related to higher levels of angiotensinogen that is associated with metabolic syndrome [6], while the I allele of ACE correlated with the development of Alzheimer's disease [7]. Moreover, the I/D polymorphism in the ACE gene has been linked to several kinds of diseases, such as coronary artery disease [8], infections in post-operative cardiac valve surgery patients [9] and arterial hypertension [10].

Therefore information of the effect I/D polymorphism on ACE activity in catalysing bradykinin is still limited. Further, we have examined the serum bradykinin level in hypertensive patients with I/D polymorphism of ACE, who were treated with ACE inhibitor. This study suggests that the polymorphism does not significantly influence the serum bradykinin level of hypertensive patients who are taking ACE inhibitors.

## 2. Subjects and methods

### 2.1. Subjects and detection of serum bradykinin level

The study recruited 64 hypertensive patients, over 18 years old, who had been taking an ACE inhibitor (lisinopril or captopril) for at least eight weeks with good medication adherence, measured by the Morisky Medication Adherence Scale (MMAS). Patients with secondary hypertension, cardiovascular diseases, liver dysfunction, and were currently receiving treatment with angiotensin receptor blockers (ARBs) were excluded from this study. A blood sample was taken to detect ACE polymorphism and measure bradykinin level. The level of bradykinin serum was measured according to the manufacturer's instructions (Cusabio Biotech; Cat. No. CSB-E09155h), the levels of bradykinin serum were examined using Analysis of Variance and quantitative variables were expressed as mean ± standard deviation values. A value of $p \leqslant 0.05$ was considered as statistically significant. This study has followed the Code of Ethics of the world Medical Association (Declaration of Helsinki) for experiments in humans and approved by Brawijaya University/RSSA hospital ethics.

### 2.2. Polymorphism detection

Genomic DNA samples were isolated by DNA extraction kit (Geneaid™). Then the genomic DNA was used as a template to amplify a DNA fragment on intron 16 of the ACE gene. The amplification was done by polymerase chain reaction (PCR), using a forward primer 5′-GCC CTG CAG GTG TCT GCA GCA TGT-3′ and reverse 5′-GGA TGG CTC TCC CCG CCT TGT CTC-3′. Thirty-four cycles of PCR were performed with the following parameters: denaturation at 96 °C for 45 s, annealing at 60.3 °C for 45 s, and extension at 72 °C for 45 s. The D allele is characterised by a 312-bp fragment, whereas 599-bp fragment indicates I allele. Each sample found to have the DD genotype was subjected to the second PCR to amplify a region inside intron 16 with insertion-specific primers 5′-TGG GAC CAC AGC GCC CGC CAC TAC-3′ and 5′-TCG CCA GCC CTC CCA TGC CCA TAA-3′ to avoid ID-DD mistyping [9].

### 2.3. Molecular docking

The ACE molecule was retrieved from Protein Data Bank in Europe (PDBe) with access code 5AMB [11]. The simplified molecular input line entry system (SMILES) structure of bradykinin and angiotensin I was taken from PubChem [12], then they were converted into PDB format using Discovery Studio Software [13]. Lisinopril and the N-domain of ACE were extracted from PDB (2C6N) [14], while captopril and the C-domain of ACE were retrieved from PDB (1UZF) [15]. Molecular docking between ACE and its ligands (lisinopril, captopril, bradykinin and angiotensin I) were performed by using AutoDock Vina, PyRx software [16]. The predicted binding affinity (kcal/mol) was calculated by AutoDock Vina. All molecules were visualised by Discovery Studio [13].

## 3. Results

### 3.1. Interaction of ACE with bradykinin and angiotensin I

ACE has two domains – the N- and C-terminals [3–5]. Both domains work independently and alternately when they catalyse their substrates [17]. ACE with *Alu* insertion (I) in the intron 16 was predicted to lose the C-domain, while the Deletion allele(D) still has two active sites [18]. Furthermore, dock-

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