RESEARCH ARTICLE

The Correlation Between Systolic Blood Pressure and Angiotensinogen Levels in Hypertensive Patients with G-6A AGT Treated With Angiotensin II Receptor Blocker: A Preliminary Study

Mohammad S. Rohman[1], Amelia Iradany[2*], Imama Maslahah[2], Mifetika Lukitasari[3], Hidayat Sujuti[4], Risa Ramadhiani[2], Akhiyan H. Susanto[3]

[1]*Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Brawijaya- Saiful Anwar General Hospital 65145, Malang, Indonesia*
[2]*Biomedical Science, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia*
[3]*School of Nursing, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia*
[4]*Biochemistry Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia*

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ABSTRACT

The existence of genetic variant -6 abnormal glucose tolerance (AGT*)* is thought to be a functional area that affects the basal transcriptional activity resulted in increased angiotensinogen levels. Single nucleotide polymorphisms (SNP) of G-6A was genotyped by using polymerase chain reaction (PCR) followed by sequencing. Serum angiotensinogen levels were measured using ELISA. In the present study, there was no statistical difference of baseline angiotensinogen levels found among genotypes. Also, there were no significant differences found in angiotensinogen levels as well as in the changes of angiotensinogen levels after a 5-month treatment of angiotensin II receptor blocker (ARB). There was also no statistically significant correlation between blood pressure and angiotensinogen levels at baseline among genotypes. However, mean SBP after 5 months of ARB treatment was found to significantly correlate with serum angiotensinogen levels in patients with AA genotype ($p = 0.05$; $r = -0.28$) but not with other genotype. However, this preliminary study should be followed by increasing the sample size to confirm the result.

Keywords : G-6A, AGT, Angiotensinogen levels, Blood Pressure, ARB.

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INTRODUCTION

The Renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure[1]. Molecular variants of the angiotensinogen gene (*AGT*), which is an important component of the RAS, are considered to be a genetic risk factor causing essential hypertension. Variation of *AGT* prometers were found in Indonesian hypertensive patients[2]. The presence of genetic variant -6 in the *AGT* promoter is considered a functional area that affects the basal transcriptional activity, resulting in increased angiotensinogen levels [3,4]. The high angiotensinogen levels are associated with increased formation of vasoactive angiotensin II and blood pressure.[5]

Our previous study has suggested the use of ARB in 42% of patients with hypertension.[6] ARB has been known to be effective in reducing blood pressure in patients with essential hypertension. If the ARB blocks RAS, a feedback mechanism will occur, followed by increased transcriptional activity of *AGT*.[7] However, the effect of ARB therapy in hypertensive patients with genetic variants G-6A remains unclear. Therefore, this study was conducted to investigate the therapeutic response in hypertensive patients with a genetic variant of G-6A *AGT* treated with ARB by measuring angiotensinogen levels and 24 hours blood pressure monitoring.

MATERIALS AND METHODS

Subjects

Fifty-six patients with primary hypertension, normal renal function (serum creatinine <1.5mg/dL), normal body mass index (18–25kg/m^2), and good medication adherence were recruited for this study. Then blood samples were taken for

**Author for Correspondence: airadany12@gmail.com*

detecting polymorphism and measuring serum angiotensinogen levels at the beginning of therapy. All patients received ARB as basal treatment, and their blood pressures were measured by ABPM at the end of the study.

Ambulatory Blood Pressure Monitoring (ABPM)

Ambulatory blood pressure monitoring was carried out using Tonoport V Firmware Version 2.1. The device was programmed to obtain blood pressure readings at 30-min intervals during the day (between 07:00 and 22:00) and at 60 minutes intervals during the night (between 22:00 and 07:00 hours). The ambulatory data used in the present study were those obtained by the oscillometric method. We excluded those who obtained less than 75% of either awake or asleep valid BP readings. During the ABPM, patients were instructed to continue their usual daily activities.

Detection of Polymorphism

Genomic DNA samples were isolated using DNA extraction kit (Geneaid™). Each sample was amplified by the following primers: 5'TTCCAGAAG CGACTTTTCAC3' and 5'TAGTACCCAGAAGAACGGCA. A 593bp region (-567 to +26) was amplified to detect four polymorphic sites in the promoter region of AGT, including at position -6 (Woodiwiss et al., 2006). The samples were then sequenced by the sequencing method (Macrogen, Korea) and the genetic variants were analyzed using Genescane software (Applied Biosystems).[8]

Measurement of Angiotensinogen Levels

A sandwich ELISA was used to measure serum angiotensinogen levels at the beginning and after 5 months of ARB treatment as previously described.[9]

Analysis

Baseline characteristics and serum angiotensinogen levels finding between 3 groups were compared by the Kruskal-Wallis test. Quantitative variables are expressed as mean ± SD values. Categorical variables were compared by the Chi-Squared test. Spearman's rho test was used for correlation analysis. For all tests, a value of $p \leq 0.05$ was considered to be statistically significant. Statistical analysis was performed under the SPSS 16.0.

Ethics

The study was approved by local on medical research ethics committee. Written informed consent was obtained from all study participants.

RESULTS

Baseline Characteristics

The baseline characteristics of hypertensive patients between 3 groups (GG, GA, AA) are shown in Table 1. Statistical analysis showed that there were no significant differences of baseline characteristics at each baseline.

Serum Angiotensinogen Levels and Genetic Variant of G-6A *AGT*

The genotype distribution frequencies were 5.3% for the GG genotype, 12.7% for the GA genotype, and 82% for the AA genotype. No statistical difference of baseline angiotensinogen levels was found between AA, GA and GG genotype ($p = 0.56$). After 5 months treatment of ARB serum angiotensinogen levels also revealed no significant difference among genotypes ($p = 0.23$), as shown in Table 2.

In order to know whether there was a significant change of serum angiotensinogen levels after treatment, post-treatment – baseline was calculated. The difference was not statistically significant as shown in Table 2 ($p = 0.40$).

Blood Pressure Profile and Serum Angiotensinogen Levels among Genetic Variant of G-6A *AGT*

As shown in Table 3, there was no statistical difference in blood pressure profile found between GG, GA and AA genotype in hypertensive patients treated with ARB. In addition, in

Table 1: Baseline characteristics of the Patients

	Genetic variant of G-6A AGT			
Variable	*GG (n = 3)*	*GA (n = 7)*	*AA (n = 46)*	*p (*)*
Age (years)	57 ± 4.2	54 ± 5.3	59 ± 8.8	0.3
Gender (M/F)	½	4/3	23/23	0.7
Ureum (mg/dl)	33 ± 10.8	30 ± 10.1	29 ± 14.0	0.6
Creatinine (mg/dl)	0.9 ± 0.2	1 ± 0.2	0.9 ± 0.3	0.1
Cholesterol (mg/dl)	183 ± 33.6	193 ± 32.3	179 ± 42.9	0.7
Blood glucose (mg/dl)	89 ± 3.0	96 ± 6.8	98 ± 17.9	0.2
BMI (kg/m^2)	27 ± 2.9	28 ± 3.9	26 ± 3.9	0.3
Antihypertensive drug combination (n,%) :				
ARB	0 (0%)	0	4 (8%)	0.6
ARB, BB	2 (67%)	2 (28%)	13 (28%)	
ARB, CCB	0 (0%)	1 (14%)	9 (19%)	
ARB, BB, CCB	1 (33%)	4 (58%)	20 (45%)	

BMI indicates body mass index ; *p value ≤0.05 = significantly different between groups

Table 2: Comparison of serum angiotensinogen levels among genetic variant of G-6A *AGT*

Angotensinogen Levels (ng/ml)	*Genetic Variant of G-6A AGT*			
	GG (n = 3)	*GA (n = 7)*	*AA (n = 46)*	*p (*)*
Pre	271 ± 111.3	324 ± 87.9	338 ± 70.2	0.5
Post	375 ± 109.1	292 ± 86.6	361 ± 100.8	0.2
Δ (delta)	104 ± 208.4	-31 ± 155.7	23 ± 120.2	0.4

*p value ≤0.05 = significantly different between groups

Table 3: Blood pressure profile in hypertensive patients according to allele

Blood Pressure Profile	*Genetic Variant of G-6A AGT*			
	GG (n = 3)	*GA (n =7)*	*AA (n = 46)*	*p value (*)*
24-h average BP (mmHg)				
SBP	131 ± 12.6	133 ± 17.0	132 ± 15.4	0.9
DBP	84 ± 14.4	87 ± 11.2	81 ± 11.1	0.4
24-h blood pressure target achievement				
Yes	2	5	34	
No	1	2	12	0.1
Daytime average BP (mmHg)				
SBP	135 ± 12.1	142 ± 22.6	138 ± 18.2	0.7
DBP	89 ± 13.2	94 ± 17.3	107 ± 15.5	0.2
Night-time average BP (mmHg)				
SBP	138 ± 9.6	147 ± 25.9	133 ± 20.1	0.2
DBP	82 ± 16.4	92 ± 18.7	80 ± .63	0.2
Dipping				
Normal	2 (3.6%)	3 (5.4%)	27 (48%)	0.7
Abnormal	1 (1.8%)	4 (7.2%)	19 (34%)	
Morning Surge				
MS	1 (33%)	1 (14%)	4 (8,7%)	0.3
non MS	2 (67%)	6 (86%)	42 (91,3%)	

BP indicates blood pressure ; SBP, systolic blood pressure ; DBP, diastolic blood pressure ; MS, morning surge ; *p value ≤0.05 = significantly different between group

order to know whether ARB therapy correlated with serum angiotensinogen levels, bivariate correlation was used. There was no statistical significance of correlation between blood pressure and angiotensinogen levels at baseline among 3 groups. Statistical analysis showed that mean SBP after 5 months treatment was found to hsignificantly correlatewith serum angiotensinogen levels, but only in ppatientswith AA genotype (p = 0.05 ; r = -0.28). However, there was no correlation found between diastolic blood pressure and angiotensinogen levels in hypertensive patients treated with ARB (Table 4).

DISCUSSION

For the first time, our present study has investigated the correlation between serum angiotensinogen levels and systolic blood pressure in Indonesian hypertensive patients treated with angiotensin II receptor blocker (ARB). At the baseline, there was no correlation found between blood pressure and serum angiotensinogen levels. However, after 5 months of ARB treatment, it was found that the systolic blood pressure correlated with increased angiotensinogen levels, especially in hypertensive patients with AA genotype. In contrast, previous studies showed no correlation between G-6A polymorphism and blood pressure response to ARB treatment. However, they used office blood pressure instead of ABPM.[10]

Other studies, both in vitro and in vivo, have shown increased baseline levels of transcription in AA genotype [11-13] On the contrary, our study has shown that there was no significant difference among genotypes, both at the baseline and after treatment, even if the changes of angiotensinogen levels after treatment in GG genotype tend to be higher compared to in other genotypes. However, the changes did not reach statistical significance. All hypertensive patients revealed decreased blood pressure after treatment regardless of genotype. Therefore, this study has shown that most hypertensive patients are responsive to 5 months of ARB treatment. However, further investigation should be conducted to see longer effect against ARB among genotypes. In addition, the increase of angiotensinogen levels should be observed further in a larger population.

In the present study, we have found the frequencies of the genotype in our population were GG (5.3%), GA (12.7%), AA (82%). Thus, the allele frequencies were G allele (5.4%) and A allele (94.6%). The high frequency of the A variant found in our population was similar to those described in the Taiwanese, Caucasian, and Chinese population.[8,14,15] Actually,

Table 4: Correlation between blood pressure and serum angiotensinogen levels among genetic variant of G-6A *AGT*

	Genetic Variant of G-6A AGT											
	GG				*GA*				*AA*			
	SBP/ DBP	*Angiotensinogen Levels*	*r*	*p value (*)*	*SBP/ DBP*	*Angiotensinogen Levels*	*r*	*p value (*)*	*SBP/ DBP*	*Angiotensinogen Levels*	*r*	*p value (*)*
Baseline	136 ± 17.7	271 ± 111.3	0.5	0.6	143 ± 23.1	324 ± 87.9	0.00	1.0	134 ± 8.3	338 ± 70.2	-0.02	0.9
	83 ± 13.3		0.5	0.6	93 ± 17.3		-0.03	0.9	84 ± 9.7		-0.22	0.1
ARB	131 ± 12.6	375 ± 109.1	0.5	0.6	133 ± 17.0	292 ± 86.6	-0.32	0.4	132 ± 15.4	361 ± 100.8	-0.28	*0.05
	84 ± 14.4		0.5	0.6	87 ± 11.2		-0.28	0.5	81 ± 11.1		-0.14	0.3

SBP, systolic blood pressure; DBP, diastolic blood pressure; r, coefficient correlation
(*) Correlation is significant at the levels 0.05 (2-tailed)

angiotensinogen levels in GG genotype largely increased after 5 months of ARB treatment. However, the level varied among individuals, resulting in a big standard deviation compared to other genotypes; there was no statistical difference. A larger sample may be able to reduce the variation. Again, levels of angiotensinogen decreased after 5 months of ARB treatment in GA genotype because wide variation seems to be not different compared to others.

Major limitation of the study was variation of additional anti hypertensive drug for all patients while they received ARB. In addition, the number of sample was relatively small. The previous study found that genetic variation at the -152 *AGT* also did not correlate with angiotensinogen levels because its samples were too small.[16] Therefore, adequate sample size is needed to analyze the statistical significance between blood pressure response and angiotensinogen levels among genotypes. It is also interesting to analyze the correlation between *AGT* mutation and angiotensinogen levels by using the multilocus study approach.

CONCLUSION

In summary, we have observed that there was a correlation between serum angiotensinogen levels and systolic blood pressure in the treatment using ARB, especially in hypertensive patients with AA genotype. However, this preliminary study should be followed by increasing the sample size to confirm the result.

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