Original article

Aldosterone levels and the -344t/c aldosterone synthase in individuals with a family history of hypertension

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Abstract:

Background: Genetic factors play an important role in the determination of hypertensive disease in a family. The -344T/C the *aldosterone synthase* gene has been reported in various populations closely related to hypertension. The aim of this study was to determine the presence of the -344T/C polymorphism of the *aldosterone synthase* gene and the levels of plasma aldosterone in individuals with and without a family history of hypertension. ***Methods:*** This study was a case control design, with healthy individuals with a family history of hypertension as cases (n-42) and those without a family history of hypertension as controls (n=41). The subjects' plasma aldosterone levels were analysed by enzyme-link immunosorbent assay (ELISA) and the gene polymorphism was analysed by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP). The data were analysed by an independent sample T-test and Chi square test, and the significance level was set at $P<0.05$. ***Result:*** The frequency of the TT + TC genotype was higher in cases, and this increase was significant different compared to the controls ($P<0.046$). The frequency of the TT + TC genotype was 2.56 higher in cases than controls. The frequency of the C allele in cases was also significantly different compared to the controls ($P = 0.039$) and 2.29 more frequent in cases than the controls ($P = 0.039$). The plasma aldosterone level was 42.35 pg/dL in cases and 34.9 pg/dL in controls ($P = 0.616$). Plasma aldosterone level in cases with the CC, TC and TT genotypes were 48.29, 40.8 and 35.2 pg/dL, respectively ($P = 0.774$). Our study concludes that individuals with a family history of hypertension are at a higher risk of developing hypertension. Follow-up studies are required to determine the incidence of hypertension in a person with a family history of hypertension and who is a carrier of genetic risk factors.

Key words: Aldosterone, Aldosterone synthase, Hypertension, Polymorphism,

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Introduction:

Hypertension is a multifactorial disease because it involves both non-genetic risk factors, such as environmental factors and common underlying risk factors, and genetic factors, or interactions between them [1]. The hypertension risk factors that can be addressed include high sodium intake, lack of physical activity, obesity, smoking, and alcohol consumption, while the risk factors that cannot be controlled include increasing age, gender, and family history of hypertension or other genetic factors [2, 3].

Genetic factors play an important role in the incidence of hypertension in a family[4]. Heredity has a significant effect on hypertension, and individuals who have a history of hypertensive parents will have a seven-fold higher risk of developing hypertension than those without a history of hypertension[5].

Gene polymorphisms play a role in the incidence of familial hypertension, which refers to hypertensive

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individuals with a family history of hypertension[6]. One of the genes that plays a role in hypertension is the *aldosterone synthase* gene (*CYP11B2* gene). The -344T/C polymorphism of the *aldosterone synthase* gene occurs in the *CYP11B2* gene promoter, and thymine (T) is substituted by cytosine (C)[7]. This allelic polymorphism of the -344T/C gene shows differential binding to the putative binding site, *steroidogenic transcription factor-1* (SF-1), thereby affecting the activity of the promoter region and changing the function and gene expression of *CYP11B2*[8,9].

Hypertension is a multifactorial disease that involves genetic and non-genetic factors. Genetic factors are an important risk factor for the incidence of hypertension in a family[4]. Individuals with parents who have hypertension have a seven-fold increased risk of developing hypertension compared with individuals whose parents are normotensive[5,6.]

The -344T/C polymorphism of the aldosterone synthase gene is a known risk factor for hypertension. In this polymorphism, the substitution of thymine (T) by cytosine (C) occurs in the promoter of the gene. The -344T/C allele binds the putative binding site of steroidogenic transcription factor-1 (SF-1), thereby altering the expression level of aldosterone synthase[8, 9]. Hypertension subjects with TT and TC genotypes have a higher aldosterone/renin ratio than those with the TT genotype[10], which contributes to the pathogenesis of hypertension.

Aldosterone levels are involved in the development of hypertension because high aldosterone levels result in excessive sodium retention[11]. The -344 T/C *aldosterone synthase* polymorphism increases the expression of *aldosterone synthase*, which leads to increased aldosterone levels in the blood. A high level of aldosterone is associated with sodium and water retention by the kidneys, which will eventually increase blood pressure[12]. The purpose of this study is to correlate the -344T/C polymorphism of the *aldosterone synthase* gene and plasma aldosterone levels in individuals with and without a family history of hypertension.

RESEARCH METHOD

This study utilized a case-control design. The subjects in this study were divided into 2 groups. The case group contained 42 subjects with a family history of hypertension who were recruited at Rajawali Citra Hospital, Yogyakarta. The control group contained 41 subjects without any family history of hypertension and were employees Rajawali Citra Hospital Yogyakarta. This study was approved by Ethical Committee Faculty of Medicine, Gadjah Mada. University.

This case-control study used 42 subjects with a family history of hypertension as cases and 41 subjects without any family history of hypertension as controls. They were Javanese who lived in Yogyakarta. The inclusion criteria for the case group were as follows: 19-39 years old, apparently healthy, have a family history of hypertension, and Javanese (at least 3 generations). The inclusion criterion for the control group was individuals without any family history of hypertension. The exclusion criteria were as follows: blood pressure $\geq$ 140/90 mmHg, obesity (body mass index $\geq$ 25 kg/m^2), pregnancy, smokers, alcohol consumption and the use of hormonal contraceptives.

The cases that met the inclusion criteria were individuals who were male or female, aged 19-39 years, apparently healthy, had a family history of hypertension, Javanese (at least 3 generations), willing to become research subjects and provided informed consent. The control group was composed of individuals who did not have any family history of hypertension. Individuals were excluded if they were diagnosed with hypertension (BP $\geq$ 140/90 mmHg) or were obese (BMI $\geq$ 25 kg/m^2), pregnant, smokers, alcohol users and hormonal contraceptive users.

The subjects' plasma aldosterone levels were analysed using ELISA (DRG kit EIA 5298), and the aldosterone synthase -344T / C *CYP11B2* gene polymorphism was determined by PCR-RFLP. DNA isolation was carried out using Wizard Genomic DNA Purification (Promega-Madison, Wi, USA) and go Taq® green. Amplification of DNA fragments of the CYP11B2 gene was carried out with the following primers: forward 5'-CAGGAGGGATGAGCAGGCAGAGCACAG-3' and reverse primer5'-CTCAACCCAGGAACCTGCTCTGGAAACATA-3'. The PCR reaction was carried out in a total volume of 30 μL, consisting of the following components: 2 μL of DNA, 15 μL PCR master mix (2x PCR buffer, dNTPs 150 mm, and 0.5 U Taq DNA polymerase), 2 μL and 11 μL H_2O.

The PCR cycling conditions were as follows: (1) initial denaturation at 94 ^oC for 5 minutes, followed by 35 cycles of PCR: (2) denaturation at 94 ^oC for 1 minute; (3) annealing at 67 ^oC for 1 minute; (4) extension at 72 °C for 1 min, (5) a final extension at 72 °C for 7 minutes, (6) cooling to 4 ^oC. The PCR program ran for 2 hours 20 minutes. Then, the PCR products were digested with the restriction

Table 2. Frequency distribution of the genotypes (TT, TC, CC) and alleles (T and C) of the -344T/C *aldosterone synthase* gene in individuals with and without a family history of hypertension, and the expectation based on the Hardy-Weinberg equation.

Variable	Case	Control	P (OR. 95% CI)	Hardy Weinberg		
				Observed	Expected	P
Genotype						
TT	23 (0.55)	31 (0.76)	0.127	54 (0.65)	53 (0.64)	0.892
TC	16 (0.38)	9 (0.22)		25 (0.30)	27 (0.32)	
CC	3 (0.07)	1 (0.02)		4 (0.05)	3 (0.04)	
Genotype						
TC+CC	19 (0.45)	10 (0.24)	**0,046**	79 (0.95)	80 (0.96)	1.00
TT	23 (0.55)	31 (0.76)	**2.56** (1.00-6.53)	4 (0.05)	3 (0.04)	
Allele						
C	22 (0.26)	11 (0.13)	**0.039**	133 (0.80)	133 (0.80)	1.00
T	62 (0.73)	71 (0.87)	**2.29** (1.03-5.10)	33 (0.20)	33 (0.20)	

enzyme HaeIII. Digestion was carried out in a final volume of 10 μL containing the following: 4 μL of PCR products, 1.0 μL of buffer, 0.5 μL (5 U) of the enzyme, and 4.5 μL of H2O to a final volume of 10 μL. The reaction mixture was incubated for 16 hours at 37 °C. The digestion products were separated by electrophoresis on a 2% agarose gel, and the products were visualized with ethidium bromide. The product was the TT genotype (wild-type) if 4 bands were observed (402 bp, 138 bp, 51 bp, and 48 bp), TC genotype if 6 bands were observed (402 bp, 344 bp, 138 bp, 58 bp, 51 bp, and 48 bp) and CC genotype if 5 bands were observed (344 bp, 138 bp, 58 bp, 51 bp, and 48 bp).

The normality of the data was tested using the

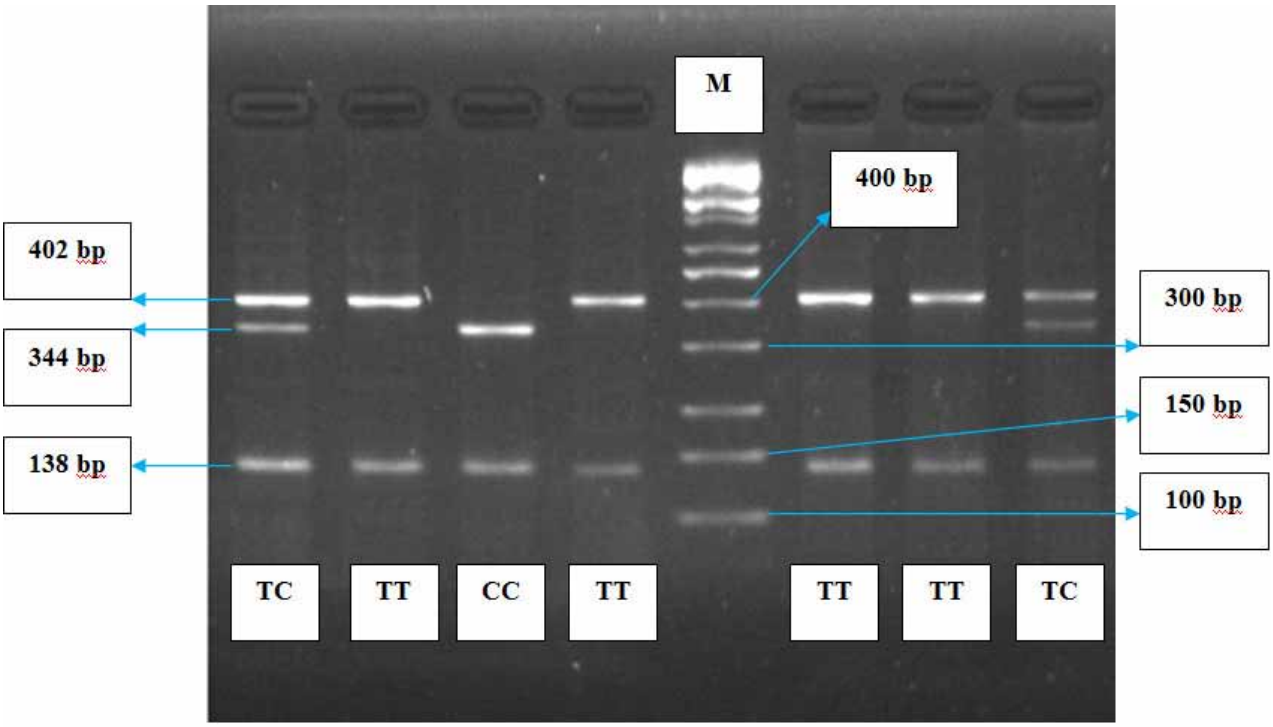

Figure 1. Results showing the PCR-RFLP products of the -344T/C *CYP11B2* gene. M = Marker; Wild-type genotype TT (402 bp, 138 bp, 51 bp, 48 bp), TC = mutant heterozygous genotypes (402 bp, 344 bp, 138 bp, 58 bp, 51 bp and 48 bp) and genotype CC = mutant homozygous (344 bp, 138 bp, 58 bp, 51 bp and 48 bp).

Table 3. Mean plasma aldosterone levels in individuals with and without a family history of hypertension based on the individuals' TT, TC and CC genotypes of the -344T/C *aldosterone synthase* gene.

	Aldosterone level (pg/dL)	P
Case (n=42)	42.35 (3.7-152.1)	0.616*
Control (n = 42)	34.9 (5.9 – 111.7)	
Genotype		
TT (n=54)	35.2 (3.7 – 119.2)	0.774**
TC (n=25)	40.8 (5.4 – 152.1)	
CC (n=4)	48.29 (28.9 – 83.0)	

*The data are reported as the median (minimum-maximum) and were analysed with the Shapiro-Wilk test.

** The data are reported as the mean ± SD or median (minimum-maximum) and were tested with the Kolmogorof Smirnov test.

Shapiro-Wilk test. Normally distributed data were assessed with the parametric test, the Independent Samples T-test. If the data distribution was not normal, they were log-transformed, and if the data still did not show a normal distribution, the Mann-Whitney U-test was used. Genotype and allele frequency differences between cases and controls were analysed by the Chi-Square test. The risk of having the C allele in the case and control groups was analysed by determining the odds ratio. The difference in the mean plasma aldosterone levels between the case and control groups was analysed with the Mann-Whitney U test, and the differences between the mean plasma aldosterone level between genotypes, TT, TC and CC, was analysed with *the Kruskal-Wallis* test. The significance limit was set at $p < 0.05$.

Results:

The case group was composed of 11 men and 31 women, and the control group was composed of 10 men and 31 women. There was no significant difference in the distribution of gender, age, body weight (BW), body height (BH), body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP) between the case and control groups (Table 1). The presence of the aldosterone synthase -344T/C gene polymorphism shown in Figure 1.

The frequency distribution of the TT, TC and CC genotypes in the case and control groups is shown in Table 2. There were no significant differences in the distribution of the genotypes between the cases and controls ($p = 0.127$). The frequency the TC + CC genotypes in the case and control groups were 0.45 and 0.24, respectively, and this difference was statistically significantly ($p = 0.046$, OR 2.56, 95% CI 1.00 to 6.53). The C allele was more frequently observed in the case group and was determined to be a risk factor for hypertension with OR 2.29 (95% CI 1.03 to 5.10). Compared to the expectations obtained with the Hardy-Weinberg equation, the frequency of genotypes and alleles observed in this study and that which was expected were not significantly different.

The aldosterone level in individuals with a family history of hypertension was higher than that in individuals without any family history of hypertension, but this difference was not statistically significant ($p = 0.616$). The mean plasma aldosterone levels in individuals with the TT, TC and CC genotypes were not significantly different ($p = 0.774$). The plasma aldosterone level higher was in subjects with the CC genotype, compared to those with the TC and TT genotypes.

Discussion:

In this study, the frequency of the TT genotype was higher in cases than in the controls. This result is consistent with other studies carried out in the Hani and Yi populations China[13] and in Japan[14]. Controversial results were obtained in populations from Brazil[8] and India[15], in that in these two populations, the frequency of the TT genotype was higher in hypertensive subjects than in normotensive subjects. The TC and CC genotypes were higher in cases than in the controls, and they were risk factors for hypertension. This result was consistent with studies carried out in Japanese [11] and Chinese populations[13]. The TC + CC genotypes were more common in hypertensive patients compared with normotensive patients. This result shows that

an individual with a family history of hypertension has a higher risk of developing hypertension. In this study, the C allele was found more frequently in cases than in the controls, which is similar to the results previously reported in minority populations (Hani and Yi) in China[13] and in a Japanese population[9]. Controversial results were found in an Indian population, and subjects with hypertension were more likely to have the C allele than normotensive individuals[15]. This study shown that the distribution of genotypes varies with ethnicity.

In this study, we found higher aldosterone levels in individuals with a family history of hypertension than the controls, although this difference was not significantly different. Another study found higher aldosterone levels in subjects with a family history of hypertension compared to those without hypertension[16]. The high aldosterone levels in subjects with a family history of hypertension may be indicative of increased blood pressure[17]. This is because a higher level of aldosterone influences the renin-angiotensin aldosterone system (RAAS), causing sodium and water retention in the kidney and ultimately resulting in an increase in blood pressure[12].

In this study, we found higher aldosterone levels in individuals with the CC genotype compared to individuals with the TC + TT genotype and in individuals with the TC + CC genotype compared to individuals with the TT genotype. However, this difference was not significantly significant. The TC and CC genotypes were found more frequently in cases than in the controls, but the aldosterone levels in the two groups were not significantly different. This result may be because in our study, cases were young, healthy adults, while in other studies, cases were hypertensive individuals. This study differs from the study in Japan[11], which showed a significantly different in the level of plasma aldosterone in the hypertensive groups. Additionally, the highest level of aldosterone was found in individuals with the CC genotype, compared with those with the TC and TT genotypes, and individuals with the TC + CC genotype had higher aldosterone levels than those with the TT genotype. Research on a population from the United States also showed different results, finding the highest levels of aldosterone in individuals with the TT genotype[18]. Dominickzac *et al*[19] found that hypertension genes contribute 20-40 % to the incidence of hypertension. The level of aldosterone is influenced by age, obesity, sodium and potassium levels, angiotensin-II and ACTH[20.21]. Additionally, Kothen *et al*[22] found that individuals aged less than 55 years old and with a family history of hypertension had a 3.8-fold higher risk of developing hypertension.

Conclusion:

In conclusion, the aldosterone synthase -344T/C gene polymorphism was more frequently found in individuals with a family history of hypertension than in those without hypertension, but the polymorphism did not influence the level of aldosterone.

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Conflict of interest: None

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