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Methylenetetrahydrofolate Reductase (MTHFR) C677T and A1298C Gene Polymorphism as Risk Factors for Essential Hypertension

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Abstract. Hypertension has relatively large morbidity and mortality rates throughout the world, including in Indonesia. The prevalence of hypertension tends to be greater in patients with a family history of hypertension. This is thought to be influenced by polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene. This study aims to determine the relationship between the polymorphism of C677T and the A1298C MTHFR gene as a risk factor for essential hypertension. An observational study with a case-control design was conducted involving 37 cases and 30 control people. Data obtained by PCR-RFLP. Data analysis was performed using chi-square and odds ratio calculations. The most common genotype for C677T polymorphism is CC (94.6%) followed by CT and TT with 2.7% each (p = 0.001) with OR of 0.099 (CI95% = 0.02-0.49). The most common genotype for the A1298C polymorphism is AC (45.9%), followed by AA (35.1%) and CC (19%) (p = 0.001). The C allele is present in 24 subjects in the case group (64.8%) and in 7 subjects in the control group (23.3%). The OR for the A1298C is 6.06 (CI 95% = 2.1-17.9). The C677T polymorphism showed statistical significance but did not modify the risk factor of essential hypertension. Whereas the A1298C polymorphism is statistically significant and has a 6-fold risk factor for essential hypertension, polymorphism A1298C Methyltetrahydrofolate Reductase (MTHFR) gene is a risk factor of essential hypertension.

Keywords: A1298C; C677T; Essential hypertension; MTHFR gene; Polymorphism

1. Introduction

Essential hypertension is a serious disease that is spread worldwide and can be a severe complication such as stroke, heart attack, retinopathy, and renal failure. Hypertension is a condition when a person's systolic blood pressure (SBP) is ≥140 mm Hg and diastolic blood pressure (DBP) is ≥90 mm Hg following repeated examination (Unger et al., 2020). According to the Indonesian Ministry of Health, hypertension can be classified into primary hypertension (90% of the case) and secondary hypertension (10% of the case (P2PTM Kementerian Kesehatan, 2019). Hypertension is the leading cause of cardiovascular diseases and the leading cause of death from stroke and ischemic heart

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disease. Hypertension is dubbed the "silent killer" because 46% of adults do not realize that they have the condition (Bell et al., 2015). West Java is currently the second-highest area with the highest prevalence in the population above 18 years of age at 34.1% (Kemenkes RI, 2018). Several studies have reported a significant correlation between non-communicable diseases, socio-demographic factors, behavior, physical condition, and history of previous sickness (Kemenkes RI, 2018). Singh et al. reported that hypertension is linked to various risk factors such as age, sex, education level, profession, area of domicile, alcohol consumption, and obesity (Singh et al., 2017).

Patients with essential hypertension often present with a wide range of symptoms, including headache, irregular heart rhythm, vision changes, chest pain, nausea, vomiting, and convulsion. Currently, essential hypertension is diagnosed by measuring blood pressure twice using a digital blood pressure monitor with an interval of 5 minutes. If the patient is confirmed for high blood pressure, a follow-up test includes electrocardiography and blood glucose levels to rule out other cardiovascular diseases and diabetes as a cause. Molecular testing for essential hypertension risk is not commonly done in Indonesia, despite the added information it can provide for identifying risk early. Therapy for essential hypertension includes diuretics, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, beta-blockers, and calcium channel blockers.

Hypertension risk factors that cannot be modified include age, sex, and genetics, while modifiable risk factors include smoking, low-fiber diet, dyslipidemia, salt over-consumption, sedentary lifestyle, stress obesity, and alcohol consumption (Kemenkes RI, 2019).

Several genes have been elucidated as a risk factor for hypertension, but genetic differences between populations greatly affect the outcome (Huang et al., 2015). Genes studied as risk factors for hypertension include the Angiotensin Converting Enzyme (ACE) gene, eNOS gene, Renin-Angiotensin System gene, etc. (Shi et al., 2021; Dhanachandra-Singh et al., 2014; Choudhury et al., 2012). In addition, the MTHFR gene is associated with cardiovascular disorders such as spina bifida, acute leukemia, nutritional deficiency and down syndrome, and premature coronary artery disease. , rheumatoid heart disease and hypertension (Ward et al., 2020; Zaghloul et al., 2019; Kedar & Chandel, 2019; Carlus et al., 2016; Wilson et al., 2013; Stover et al., 2015; Cortese & Motti, 2001; Wiemels et al., 2001)

Methylenetetrahydrofolate reductase is an enzyme in the methyl cycle expressed by the MTHFR gene. This gene is located in chromosome 1p36.3 at the base pair of 11.785.730-11.806.103.

Methylenetetrahydrofolate reductase catalyzes 5,10-methylenetetra hydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocvsteine re-methvlation to methionine. Polymorphism in MTHFR is suspected to be one of the causes of elevated homocysteine, which can lead to increased blood pressure (Leclerc et al., 2013). Genetic research to see the genetic contribution to the phenotype of hypertension cases so that it can have implications for management (Padmanabhan et al., 2015). In addition, it is also known that internal factors such as genetics contribute to the occurrence of primary hypertension. Polymorphisms in hundreds of genes are associated with risk factors for hypertension (Evangelou et al., 2018). This research analyzes the possible involvement of MTHFR C677T and A1298C polymorphisms with essential hypertension. Both polymorphisms have been demonstrated to be involved in developing essential hypertension in various populations but are unknown in the Indonesian population. Genetic markers for essential hypertension can help first-line screening for risk factors in disease development.

2. Methods

2.1. Patient Selection

An analytical study with a case-control design was conducted in the medical faculty of Swadaya Gunung Jati University, Indonesia, to assess the association between Methyltetrahydrofolate Reductase (MTHFR) C677T And A1298C gene polymorphism and essential hypertension. The target population was essential hypertensive patients. We used the purposive sampling method.

The inclusion criteria were all diagnosed with essential hypertensive and ECG normal. Subjects with the following condition were excluded, i.e.: (1) patients with any secondary cause of hypertension; (2) patients with abnormal ECG (4) patients who disagreed with giving blood for the study. Control subjects with the following condition, i.e.: (1) patient with normal blood pressure; (2) patient with normal ECG. (Figure 1) This study was approved by the Ethical Committee of the Faculty of Medicine, Universitas Swadaya Gunung Jati, Cirebon, Indonesia. All patients had signed written informed consent prior to the study. Written informed consent was obtained from all participants prior to enrolment. A written consent was obtained from their parents or guardians for underaged patients. The Institutional Review Board of Faculty of Medicine Universitas Swadaya Gunung Jati, Cirebon, Indonesia, approved the study protocol and followed the ethical principles of the Declaration of Helsinki of 1975 and its revision.

2.2. Blood Collection & DNA Extraction

Blood samples were collected from the peripheral vein. Then, they were put in EDTA-coated tubes and kept cold. QIAamp kit (Qiagen, Tokyo, Japan) extracted the DNA from leukocytes according to the standard protocol.

2.3. Genetic Analysis

PCR-RFLP for C677T with forward primers 5'TGAAGGAGAAGGTGTCTGCGGGA3' and reverse 5'AGGACGGTGCGGTGAG AGTG3', A1298C with forward primer 5'CAAGGAGGAGCTGCTGAAGA3' and reverse 5'CCACTCCAGCATCACTCACT3'. PCR was conducted using the following settings: C677T initial denaturation at 95 °C for 5 min. Thirty-five cycles of denaturation at 94°C for 30 sec; annealing at 60°C for 30 sec, extension at 2°C for 30 sec, and final extension at 2°C for 5 min. PCR settings for A1298C: initial denaturation at 94°C for 4 min, 30 cycles of denaturation at 94°C for 60 sec, annealing at 60°C for 60 sec, extension at 2°C for 60 sec, and final extension at 2°C for 10 min. The C677T PCR product was then digested using Hinfl and visualized with 1% agarose gel. The Al298C PCR product was subsequently digested using MboII and visualized with 1% agarose gel (Pratamawati et al., 2018).

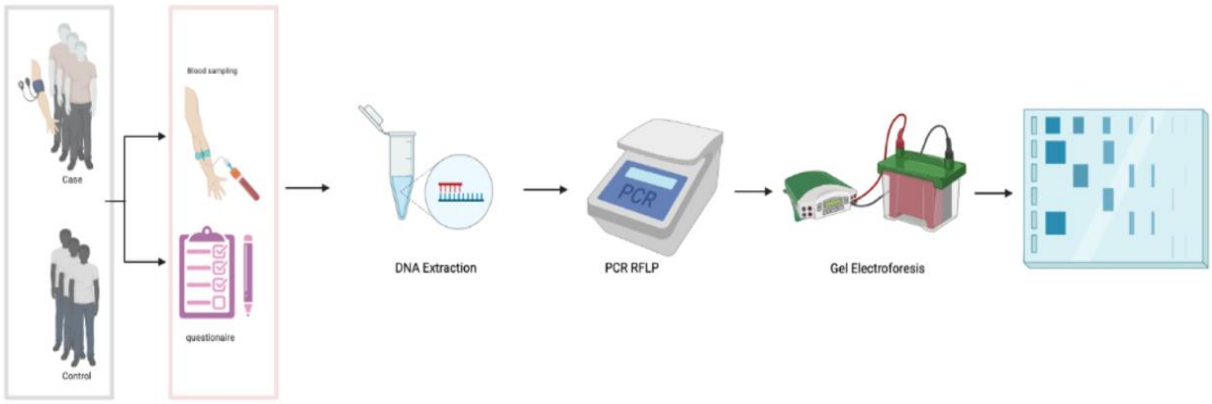

Figure 1 Work-flow schematics

3. Results and Discussion

This research recruited 34 male patients and 33 female patients, with an average age in the subject's age of 40.30 years old and in the control group of 40.50 years (Table 1). MTHFR C677T genotype distribution in the case group is CC (n=35, 94.6%), CT (n=1, 2.7%), and TT (n=1, 2.7%), and in the control group CC (n=19, 63.4%), CT (n=10, 33.3%), and TT (n=1, 2.7%). The distribution of the C allele is 71 (95.9%) in the case group and 48 in the control group (80%), while the T allele distribution is 3 (4.1%) in the case group and 12 (20%) in the control group (Table 2).

MTHFR A1298C in the case group is AA (n=13, 35.1%), AC (n=17, 45.9%), and AC (n=7, 19%), and in the control group AA (n=23, 76.7%), AC (n=13.3%), and CC (n=3, 10%). The distribution of the A allele is 43 (58.1%) in the case group and 50 in the control group (83.3%). The distribution of the C allele in the case group is 43 (58.1%) and 50 in the control group (83.3.%).

Statistical analysis showed significant relations between MTHFR C6777T with essential hypertension but not a risk factor of essential hypertension (p=0.001, OR=0.099), while MTHFR A1298C showed statistical significance with essential hypertension and also a risk factor of essential hypertension (p=0.001, OR=6.06) (Table 3).

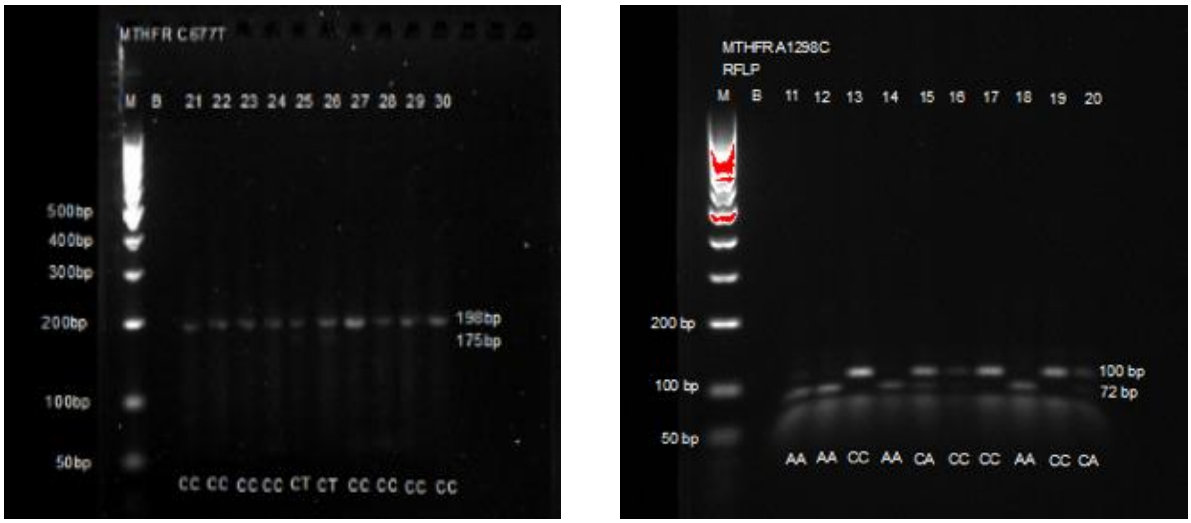

Figure 2 (Left to Right) Agarose gel of MTHFR C677T polymorphism PCR-RFLP product, digestion result (CC: 198 bp; CT: 198 bp, 175 bp; TT: 175 bp), B: Blank, M: Marker, (b) Al298C RFLP result, M: Mark, B: Blank, digestion result (AA: 72 bp; AC: 100 bp and 72 bp; CC: 100 bp)

Table 1 Demographic data of participants

Characteristics	Case (n = 37)	Control (n = 30)	p-values
Age			
At collection	40.30	40.50	0.070
Sex			
Men	22 (59.5%)	18 (60%)	
Women	15 (40.5%)	12 (40%)	

There is no significant difference in the average age between the case and control groups with similar percentages between male and female subjects in both groups.

Table 2 Allelic and genotype distribution for MTHFR C677T dan A1298C

Variables	Essential Hypertension (+)	Essential Hypertension (-)
MTHFR C677T genotype		
CC	35 (94.6%)	19 (63.4%)
CT	1 (2.7%)	10 (33.3%)
TT	1 (2.7%)	1 (3.3)
Allele		
C	71 (95.9%)	48 (80%)
T	3 (4.1%)	12 (20%)
MTHFR A1298C genotype		
AA	13 (35.1%)	23 (76.7%)
AC	17 (45.9%)	4 (13.3%)
CC	7 (19%)	3 (10%)
Allele		
A	43 (58.1%)	50 (83.5%)
C	31 (41.9%)	10 (16.7%)

Table 3 MTHFR C677T dan A1298C polymorphisms and essential hypertension

Variables	Hypertension (+)	Hypertension (-)	OR	p-values
MTHFR C677T				
Polymorphism	2 (5.4%)	11 (36.7%)	0.099	0.001
Wild Type	35 (94.6%)	19 (63.3%)	1	
MTHFR A1298C				
Polymorphism	24 (64.9%)	7 (23.3%)	6.06	0.001
Wild Type	13 (35.1%)	23 (76.7%)	1	

In the case group, 64.9% subjects with A1298C MTHFR polymorphism, with only 23.3% in the control group showing a statistically significant relation between A1298C polymorphism and a risk factor for essential hypertension with an odds ratio six times higher than subjects without the polymorphism (p=0.001). In contrast, only two subjects (5.4%) in the case group have the C677T MTHFR polymorphism compared to 11 subjects (36.7%) in the control group with odds ratio <1, meaning that the polymorphism is not statistically significant as a risk factor for essential hypertension.

This result is different compared to the previous MTHFR C677T research by Candrasarta in 2010 on 213 patients and 202 controls in Indonesia. The research showed a statistical significance p-value of 0.001 and OR of 2.1. The difference in results can probably be attributed to the sample size that made this research statistically significant but not as a risk factor (Candrasatria et al., 2020). The result in this study is similar to previous studies in MTHFR C677T and A1298C in mothers having children with Down syndrome, where the result is not statistically significant for C677T polymorphism but statistically significant for A1298C (Pratamawati et al., 2018). Other research regarding MTHFR A1298C in Indonesian rheumatoid heart disease showed significant results (Nauphar et al., 2019). The results of this study are also in line with the research of Rochmah et al. (2018), there was a significant relationship between MTHFR A1298C and no relationship with C677T in non-syndromic cleft lips/palate cases in the Indonesian Sasak tribe (Rochmah et al., 2018).

Table 4 MTHFR C677T polymorphisms in different populations

First Author	Year	Country	Ethnicity	Diagnostic	genotype		
					Case	Control	P value
Fridman (Fridman et al., 2013)	2013	Argentina	White	SBP >140 DBP > 90	CC: 29 CT: 40 TT: 6	CC: 71 CT: 64 TT: 15	0.917
Yin (Yin et al., 2012)	2012	China	Asian	SBP >140 DBP > 90	CC: 244 CT: 358 TT: 68	CC: 322 CT: 309 TT: 51	0.047
Nakata (Nakata et al., 1998)	1998	Japan	Asian	SBP >160 DBP > 95	CC: 63 CT: 91 TT: 19	CC: 65 CT: 83 TT: 36	0.309
Deshmukh (Deshmukh et al., 2009)	2009	United States	White	SBP >140 DBP > 90	CC: 22 CT: 16 TT: 4	CC: 52 CT: 48 TT: 28	0.221

Abbreviations: SBP: Systolic blood pressure; DBP: Diastolic blood pressure

We can see the difference between the C667T polymorphisms MTHFR gene in hypertension in different populations, while MTHFR A1298C polymorphism studies are still rarely done in hypertension.

4. Conclusions

MTHFR C677T polymorphism is statistically significant with essential hypertension but is not a risk factor in essential hypertension. In contrast, MTHFR A1298C is also statistically significant inessential hypertension and is a risk factor in essential hypertension. Individuals with A1298C polymorphism have a six times increased risk of developing essential hypertension. Findings from this research can be used for further research, such as haplotype analysis or downstream analysis with next-generation sequencing for the MTHFR gene.

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