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Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism rather than homocysteine increase the risk of ischemic stroke-associated executive dysfunction

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ABSTRACT

Background: An executive dysfunction is a form of cognitive impairment commonly found in ischemic stroke patients with a significant impact on the patients' quality of life. The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and homocysteine are considered important risk factors for stroke and various forms of cognitive dysfunction. This study aimed to investigate the association between the MTHFR C677T polymorphism and serum homocysteine levels and the risk of ischemic stroke-associated executive dysfunction.

Method: 87 ischemic stroke patients within the first three months of stroke onset in West Nusa Tenggara General Hospital, Hajar Hospital, and Mataram General Hospital were recruited. Serum homocysteine levels were assessed using ELISA. The MTHFR C677T polymorphism was analyzed using the PCR-RFLP procedure. The executive function of the patients was evaluated using Trail Making Test Part-B, verbal fluency, and backward digit span tests.

Results: The mean age of the subjects was 54.1 years. CT scan data revealed that 78.2% of subjects had a small infarct size. The MTHFR C677T polymorphism frequency was 25.3% consisting of 2.3% homozygous and 23.0% heterozygous mutants. Multivariate logistic regression analysis showed that patients with MTHFR C677T polymorphism were more likely to have executive dysfunction than those with the wild-type genotype of the MTHFR gene (OR 4.53, 95% CI 1.06–19.27, p = 0.041). However, patients with hyperhomocysteinemia (≥13 µmol/l) were not associated with an increased risk of executive dysfunction.

Conclusion: The MTHFR C677T polymorphism was the risk factor for ischemic stroke executive dysfunction.

Keywords: stroke, executive dysfunction, MTHFR C677T polymorphism, homocysteine.
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INTRODUCTION

An executive dysfunction is a form of cognitive dysfunction with a high frequency in ischemic stroke patients. The prevalence of executive dysfunction in ischemic stroke patients is in the range of 49.5 - 64.4%.[1,2] Compared with dysfunction in other cognitive domains, executive dysfunction causes a more significant decrease in quality of life in ischemic stroke patients.[3] The decline in quality of life is related to the decreased ability of stroke patients in problem-solving, planning and initiating an activity, adapting to new situations, and modifying previously prepared plans if needed in their daily functional and social activities.[4] Although ischemic stroke-associated executive dysfunction can develop in the early phase of ischemic stroke, adequate cognitive rehabilitation intervention in this condition may provide optimal clinical outcomes.[5] It suggests that early identification of risk factors that might predict the development of executive dysfunction in the early phase of ischemic stroke is important.

The results of previous studies have shown inconsistent relationships between vascular risk factors and cognitive dysfunction associated with ischemic stroke, including executive dysfunction.[6,7] However, this investigation is still ongoing as part of efforts to develop prevention and intervention strategies for ischemic stroke-associated executive dysfunction, including studies at the biomarker and genetic level. Homocysteine is considered an important biomarker for risk assessment of ischemic stroke and Alzheimer's disease.[8,9] This is related to its ability to induce oxidative stress, neuroinflammation, and glutamate excitotoxicity, a series of pathological processes found in stroke-related cognitive dysfunction.[10] Hyperhomocysteinemia is associated with the development of white matter hyperintensity in the frontal region, a brain structure responsible for executive function, suggesting its possibility as a risk factor for ischemic stroke-associated executive dysfunction.[11]

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that plays an important role in regulating

homocysteine levels in the body through its action by converting homocysteine to methionine via a folate-dependent remethylation pathway.[12] The MTHFR C677T polymorphism, one of the polymorphisms of the gene encoding this enzyme, is considered to be one of the important genetic risk factors for ischemic stroke and dementia.[13] Previous studies showed that the MTHFR C677T gene polymorphism among ischemic stroke patients was relatively high, including in the Indonesian population, ranging from 13.9-40.3% for the CT genotype and 3.2-7.0% for the TT genotype.[14–16] In general, the variation in the prevalence of these gene polymorphisms is strongly influenced by ethnicity. This gene polymorphism will decrease the MTHFR enzyme activity at 37°C or higher and increase blood homocysteine levels.[17] Similar to hyperhomocysteinemia, the MTHFR C677T polymorphism is also a risk factor for the development of white matter hyperintensity, a pathology in the brain previously described to be associated with the risk of executive dysfunction.[18] However, there are currently no reported data regarding the association between the MTHFR C677T gene polymorphism and the prevalence of executive dysfunction among ischemic stroke patients. Therefore, this study aimed to investigate whether MTHFR C677T polymorphism and serum homocysteine levels are associated with the risk for executive dysfunction in ischemic stroke patients.

METHODS

Research design and participants

This cross-sectional study enrolled 87 ischemic stroke outpatients recruited consecutively in three hospitals in Mataram City, Indonesia, including West Nusa Tenggara General Hospital, Siti Hajar Hospital, and Mataram General Hospital from November 2019 to October 2020. The inclusion criteria for the subjects were those with a confirmed ischemic stroke diagnosis by head CT scan within the first three months of stroke onset, aged 40 – 70 years, having a minimum 6-year-education, and fully conscious. The exclusion criteria applied in this study were the presence of significant visual and hearing loss, history of diagnosis of dementia and psychiatric disorders before stroke onset. All of the subjects provided written informed consent before participation. Ethical approval was obtained from Ethical Committee for Health Research, Faculty of Medicine, Universitas Mataram (Register number: 1077/UN4.6.4.5.31/PP36/2019).

Data collection

Socio-demographic and clinical data collected from the subjects included age, sex, occupation, years of education, stroke characteristics (infarct size and side of the lesion in the brain), and vascular risk factors (cigarette smoking status, hypertension, diabetes, dyslipidemia, overweight/obesity, and atrial fibrillation). Infarct size was defined as the diameter of the infarct on the CT scan of the head and categorized as small (<15 mm in diameter) and larger (≥15 mm in diameter) infarct size.[18] The side of the lesion in the brain was categorized as left, right, and bilateral. Overweight/obesity is defined as the result of measuring the body mass index (BMI) of the subject that shows ≥25 kg/m^2.[19] Data regarding cigarette smoking status, hypertension, diabetes, dyslipidemia, and atrial fibrillation were obtained from the information provided by the subjects, caregivers, and their medical records.

Assessment of serum homocysteine

The serum samples used for homocysteine assay were extracted from three milliliters of 12 hours fasting blood samples by centrifugation at 3000g for 10 minutes and stored at -80°C until assay. Serum homocysteine levels were assessed quantitatively using an enzyme-linked immunoassay (ELISA) kit (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd., China, Catalog No: E3292Hu) according to manufacturer instructions. A serum homocysteine level of ≥13 µmol/l was categorized as hyperhomocysteinemia.

Assessment of MTHFR C677T gene polymorphism

Genomic DNA was isolated from 200 µl anticoagulant-added whole blood using a DNA isolation kit (TIANamp Genomic DNA Kit, TIANGEN Biotech (Beijing) Co., Ltd., China, Catalog No: DP304) according to the protocols provided by the manufacturer. MTHFR C677T polymorphism was analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) procedure. A total volume of 12.5 µl of PCR mixture containing 1 µl of genomic DNA, 1 µl of forward (sense) primer (5'- TGAAGGAGAAGGTGTCT GCGGGA-3'), 1 µl of reverse (antisense) primer (5'-AGGACGGTGC GGTGAGAGTG-3'), 6.25 µl of 2xTaq Plus PCR MasterMix (TIANGEN Biotech (Beijing) Co., Ltd., China, Catalog No: KT205), and 3.25 µl of ddH2O was used for DNA amplification. DNA amplification using PCR (Thermal cycler Applied Biosystem 2720) was carried out in 3 stages, including a cycle of predenaturation at 94°C for 3 minutes, denaturation at three different temperatures (30 cycles at 94°C for 30 seconds, 30 cycles at 62°C for 30 seconds, and 30 cycles at 72°C for 1 minute), and a cycle of extension at 72°C for 5 minutes.

The PCR products were digested with HinfI (New England Biolabs, USA, Catalog No: R0155T). A total volume of 20 µl of reaction mixture containing 7 µl of PCR products, 0.5 µl of HinfI, 2 µl of buffer R, and 10.5 µl of ddH2O was incubated at 37°C for 3 hours. The PCR fragments resulting from HinfI activity during the incubation period were then electrophoresed on a 3% agarose gel for 10 minutes at 100 Volt. The MTHFR gene with wild type (CC) genotype produced a single fragment of 198 bp. However, the MTHFR gene with mutant heterozygous (CT) genotype produced 2 fragments, i.e. 198 bp and 175 bp, respectively, and one with mutant homozygous (TT) genotype produced a single fragment of 175 bp. Nine PCR products of the subjects were selected for DNA sequencing analysis to verify PCR-RFLP results. This analysis was conducted in 1st BASE DNA Sequencing Service, Singapore.

Assessment of executive function

The executive function status of the subjects was assessed by performing Trail Making Test Part-B (TMT-B), verbal fluency and backward digit span (BDS) tests, the most widely used combination of tests for this cognitive domain assessment. The TMT-B was performed by asking the subjects to connect the numbers and letters

circled on a sheet of paper, starting from the smallest to the largest number/letter. A verbal fluency test was done by asking the subjects to mention the name of animals as many as they could within 60 seconds. The BDS test was conducted by asking the subjects to recall a series of numbers read to them but in backward sequence. The cut-off values for the normal results of the three tests were the completion time of ≤ 180 seconds for the TMT-B test, the ability to mention ≥ 14 animal names in 1 minute for the verbal fluency test, and the ability to recall ≥ 4 numbers in reverse order for BDS.[20,21] Subjects showing two or more abnormal results on the tests mentioned above were considered to have executive dysfunction.[19]

Statistical analysis

Socio-demographic and clinical data of the subjects were presented as mean value ± standard deviation (SD) for continuous variables normally distributed, median (minimum-maximum) for continuous variables not normally distributed, or frequency (%) for categorical variables. The data distribution was analyzed using a one-sample Kolmogorov-Smirnov test. The significant difference in the medians of homocysteine levels between mutant (CT/TT) and wild-type (CC) genotypes of MTHFR gene groups was analyzed using the Mann-Whitney U test. The crude odds ratios (OR) with a 95% confidence interval (CI) of MTHFR C677T polymorphism and homocysteine levels associated with executive dysfunction were examined using bivariate analysis. The contribution of MTHFR C677T polymorphism and homocysteine levels to executive dysfunction were examined using multivariate logistic regression analysis after controlling covariates, including socio-demographic variables (age, gender, years of education, and occupation) for Model 1; plus stroke characteristics (infarct size and side of the lesion in the brain) for Model 2; plus identified vascular risk factors (cigarette smoking, hypertension, diabetes, dyslipidemia, overweight/obesity, and atrial fibrillation) for Model 3 and reported as adjusted OR with 95%CI. The analysis was performed using SPSS 22.0 statistical software and statistical significance was set at $p<0.05$.

RESULTS

Table 1 presents the socio-demographic and clinical characteristics of ischemic stroke subjects (n=87). The mean (SD) age of the subjects was 54.1 (7.0) years; 67 (77%) were young adults; 63 (72.4%) were male; 28 (32.2%) were highly educated; and 59 (67.8%) were active workers. Based on head CT scan, 68 (78.2%) had small infarct size and there was comparable involvement of the left and right brain hemispheres, 35 (40.2%) and 34 (39.1%), respectively. Hypertension and dyslipidemia were the most commonly identified vascular risk factors, 79 (90.8%) and 53 (60.9%). Most importantly, the prevalence of the MTHFR C677T polymorphism was 25.3% consisting of 2.3% homozygous mutants (TT) and 23.0% heterozygous mutants (CT); the frequency of hyperhomocysteinemia was 9 (10.3%); and the frequency of executive dysfunction was 42 (48.3%). The frequency of homozygous mutant (TT), heterozygous mutant (CT), and wild type (CC) genotypes was based on the results of polymorphism analysis using gel

Table 1. Socio-demographic and clinical characteristics of ischemic stroke patients, n=87

Variables	Frequency (%)/ mean±SD/ median (range)
Age in years, mean±SD	54.1±7.0
Age groups, n(%)	
Young adults	67 (77.0)
Olders	20 (23.0)
Male, n(%)	63 (72.4)
Years of education groups, n(%)	
Highly educated (>12 years)	28 (32.2)
Low education (≤12 years)	59 (67.8)
Workers, n(%)	59 (67.8)
Infarct size, n(%)	
Larger (≥15 mm in diameter)	19 (21.8)
Small (<15 mm in diameter)	68 (78.2)
Side of lesion, n(%)	
Left hemisphere	35 (40.2)
Right hemisphere	34 (39.1)
Bilateral	18 (20.7)
Cigarette smoking, n(%)	34 (39.1)
Hypertension, n(%)	79 (90.8)
Diabetes, n(%)	33 (37.9)
Dyslipidemia, n(%)	53 (60.9)
Overweight/obesity, n(%)	34 (29.1)
Atrial fibrillation, n(%)	7 (8.0)
Genotype of MTHFR C677T, n(%)	
Homozygous mutant (TT)	2 (2.3)
Heterozygous mutant (CT)	20 (23.0)
Wild-type (CC)	65 (74.7)
Serum Hcy level in µmol/l, median (min – max)	2.4 (0.47 – 36.92)
Hyperhomocysteinemia (≥13 µmol/l), n(%)	9 (10.3)
Executive dysfunction, n(%)	42 (48.3)

Table 2. Association between MTHFR C677T polymorphism and serum homocysteine level in ischemic stroke patients, n=87

MTHFR C677T	n	Homocysteine (µmol/l)	p-value
CT/TT	22	3.12 (1.97 – 36.92)	<0.001
CC	65	2.34 (0.47 – 13.34)	

electrophoresis on PCR-RFLP products (Figure 1). The results of DNA sequencing also confirmed these findings.

Table 2 presents the result of the Mann-Whitney U test in investigating the association between the MTHFR C677T polymorphism and serum homocysteine levels. The result showed that the median serum homocysteine levels of subjects with the mutant (CT/TT) genotype was significantly higher than those with the wild-type (CC) genotype (p<0.001).

Table 3 presents the bivariate analysis results in examining the relationship between the MTHFR C677T polymorphism and serum homocysteine levels and the frequency of executive dysfunction. Subjects with the mutant (CT/TT) genotype of the MTHFR gene were more likely to have executive dysfunction than those with the wild-type (CC) genotype (OR 3.02, 95% CI 1.08 – 8.40, p = 0.035). Subjects with hyperhomocysteinemia were also more likely to have executive dysfunction than those with normal serum homocysteine levels (OR 10.35, 95% CI 1.24 – 86.81, p = 0.031).

Table 4 presents the multivariate logistic regression analysis results in investigating whether the MTHFR C677T polymorphism and serum homocysteine levels independently increased the risk of developing executive dysfunction associated with ischemic stroke after adjustment by controlling for other factors and independent variables. In Models 1 and 2, neither the MTHFR C677T polymorphism nor higher serum homocysteine levels were associated with ischemic stroke-associated executive dysfunction. However, in Model 3, after controlling for all independent variables (socio-demographic, stroke characteristics, and identified vascular risk factors), subjects with the MTHFR C677T polymorphism were 4.53 times more likely to have ischemic stroke-associated executive dysfunction compared to those with the wild-type genotype of MTHFR gene (OR 4.53, 95% CI 1.06 – 19.27, p = 0.041). At the same time, hyperhomocysteinemia alone was not associated with an increased risk for ischemic stroke-associated executive dysfunction. Still in Model 3, the risk of ischemic stroke-associated executive dysfunction was also increased by other independent risk factors, including low level of education (OR 5.69, 95% CI 1.40 – 23.06, p = 0.015), active cigarette smoking (OR 4.86, 95% CI 1.08 – 21.87, p = 0.039), and overweight/obesity (OR 5.37, 95% CI 1.05 – 19.22, p = 0.010).

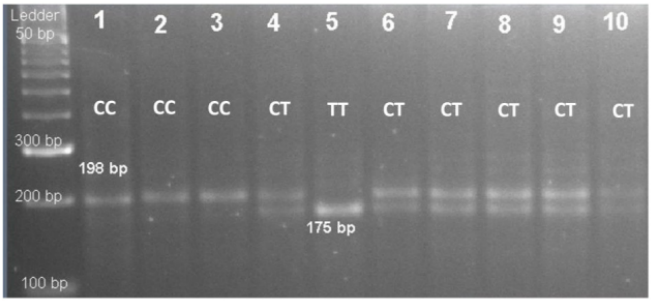

Figure 1. The results of polymorphism analysis using gel electrophoresis on PCR-RFLP products from several study samples. The CC genotype (wild-type) of the MTHFR gene showed a fragment of 198 bp, the CT genotype (heterozygous mutant) showed two fragments of 198 bp and 175 bp, and the TT genotype (homozygous mutant) showed a fragment of 175 bp. PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; MTHFR: methylenetetrahydrofolate reductase.

Table 3. Bivariate analysis between MTHFR C677T polymorphism and serum homocysteine level with executive status in ischemic stroke patients, n=87

Variables	Executive function status		OR (95% CI)	p-value
	Normal (n=47)	Dysfunction (n=40)		
The genotype of MTHFR C677T				
CT/TT	7 (31.8)	15 (68.2)	3.02 (1.08 – 8.40)	0.035
CC	38 (58.5)	27 (41.5)	Ref	
Homocysteine				
≥13 µmol/l	1 (11.1)	8 (88.9)	10.35 (1.24 – 86.81)	0.031
<13 µmol/l	44 (56.4)	34 (43.6)	Ref	

DISCUSSION

This initial study aimed to investigate the association between the MTHFR C677T polymorphism and serum homocysteine levels and the risk of ischemic stroke-associated executive dysfunction. Based on previous studies, executive dysfunction is one of the most common cognitive domain disorders found in ischemic stroke. This study demonstrated that the prevalence of MTHFR C677T polymorphism in ischemic stroke subjects was 25.3% consisting of 2.3% homozygous mutants (TT) and 23.0% heterozygous mutants (CT). This result is in line with previous studies conducted in ischemic stroke patients in Semarang and Medan, which showed the prevalence of the MTHFR C677T gene polymorphism was in the range of 3.8-4.2% for the TT genotype and 13.9-40.3% for the CT genotype.[15,16] This study also revealed that the risk of ischemic stroke-associated executive dysfunction was increased in the presence of mutant (CT/TT) genotypes of the MTHFR gene but not in hyperhomocysteinemia. Our study is the first study that showed that MTHFR C677T polymorphism contributes to an increased risk of developing executive dysfunction in ischemic stroke patients. Since most previous studies have tended to explore the relationship between MTHFR polymorphisms and global cognitive function in stroke patients, whereas cognitive rehabilitation generally has high success when targeted specifically at the impaired domain, the results of this study

are important to add to previous data.

This study also demonstrated that although the MTHFR polymorphism was significantly associated with serum homocysteine levels, elevated homocysteine levels did not increase the risk of stroke-related executive dysfunction, suggesting a possible distinct mechanism of the MTHFR C677T polymorphism in the development of this executive dysfunction, other than through the induction of hyperhomocysteinemia. A decrease in SAM levels, especially in areas of the brain that play an important role in carrying out cognitive function, may be proposed as one of the mechanisms that could explain these findings.[13] Since the enzyme MTHFR is also needed in facilitating the release of methyl groups from folate during the homocysteine remethylation process to finally produce SAM, a decrease in the enzymatic activity of MTHFR is caused by the MTHFR C677T gene polymorphism will potentially cause a decrease in SAM levels.[22] Since SAM is a universal methyl donor required for DNA methylation processes that are important for the downregulation of pathological genes of various neurodegenerative diseases, this decrease in SAM levels will eventually lead to DNA demethylation of pathological genes that result in the activation of these genes and the development of neurodegenerative diseases and cognitive decline, a process known as epigenetic mechanisms.[23] So far, there is no direct evidence to suggest that decreased levels of SAM also found in stroke. However, previous studies have shown that plasma S-adenosylhomocysteine (SAH), a product of the methyltransferase reaction involving SAM, is elevated in patients with vascular disease, including stroke. These results suggest that an increase in SAH levels in vascular disease indirectly indicates a decrease in SAM levels.[24,25] However, this potential mechanism still requires confirmation through further investigation.

Figure 2. **The results of DNA sequencing of the MTHFR gene were presented using the BioEdit computer program.** A. DNA sequence of homozygous (TT) genotype of MTHFR gene. B. DNA sequence of heterozygous (CT) genotype of MTHFR gene. C. DNA sequence of wild-type (CC) genotype of MTHFR gene. MTHFR: methylenetetrahydrofolate reductase.

The MTHFR C677T gene polymorphism is a form of missense mutation is characterized by a substitution of cytosine (C) to thymine (T) at the nucleotide position 677 on exon 4 of the MTHFR gene (at the nucleotide position 11,796,321 according to GRCh38).[26] Structurally, these mutations cause the substitution of the amino acid alanine to valine in the resulting MTHFR enzyme.[17] The change in the amino acid product from natural to valine occurs in the N-terminal catalytic domain of the MTHFR enzyme which decrease in the enzymatic activity of the MTHFR enzyme up to 30-60% of normal activity.[27]

This study also indicated that years of education were the only socio-demographic characteristic statistically significant in increasing the risk of ischemic stroke-associated executive dysfunction. This result is in line with the results of the previous study.[28,29] During the early post-stroke period, there is a reorganization of

Table 4. Multivariate logistic regression analysis of independent variables associated with executive dysfunction in ischemic stroke patients, n=87

Independent variables	OR	95% CI	p-value
Model 1			
MTHFR C677T	2.77	0.85 – 9.06	0.091
Homocysteine	7.64	0.78 – 74.94	0.081
Age	1.65	0.44 – 6.15	0.457
Sex	1.88	0.53 – 6.58	0.327
Occupation	2.04	0.58 – 7.16	0.263
Years of education	3.34	1.04 – 10.72	0.043
Model 2			
MTHFR C677T	2.54	0.72 – 8.89	0.146
Homocysteine	8.75	0.82 – 93.74	0.073
Age	1.79	0.47 – 6.82	0.394
Sex	1.87	0.52 – 6.77	0.340
Occupation	2.08	0.57 – 7.58	0.266
Years of education	3.17	0.97 – 10.34	0.056
Infarct size	0.89	0.26 – 2.99	0.845
Left vs. right hemisphere (side of lesion)	0.76	0.19 – 3.04	0.702
Bilateral vs. right hemisphere (side of lesion)	1.17	0.30 – 4.52	0.801
Model 3			
MTHFR C677T	4.53	1.06 – 19.27	0.041
Homocysteine	4.69	0.44 – 50.45	0.202
Age	2.04	0.44 – 9.43	0.359
Sex	0.75	0.14 – 3.94	0.738
Occupation	1.72	0.39 – 7.56	0.476
Years of education	5.69	1.40 – 23.06	0.015
Infarct size	0.92	0.24 – 3.51	0.909
Left vs. right hemisphere (side of lesion)	0.51	0.11 – 2.35	0.385
Bilateral vs. right hemisphere (side of lesion)	0.80	0.17 – 3.67	0.771
Cigarette smoking	4.86	1.08 – 21.87	0.039
Hypertension	1.70	0.25 – 11.40	0.583
Diabetes	0.89	0.26 – 2.99	0.847
Dyslipidemia	0.98	0.30 – 3.19	0.985
Overweight/obesity	5.37	1.05 – 19.22	0.010
Atrial fibrillation	0.30	0.03 – 2.73	0.285

Model 1: adjusted for homocysteine, age, sex, occupation, and education; Model 2: Model 1 plus stroke characteristics (infarct size and side of lesion); Model 3: Model 2 plus identified vascular risk factors (cigarette smoking, hypertension, diabetes, dyslipidemia, overweight/obesity, and atrial fibrillation).

the neuronal circuits disrupted by ischemic events and allows compensation for the cognitive decline of the affected brain area so that cognitive function remains intact, a mechanism known as post-stroke neuroplasticity.[30,31] Level of education, one of the indicators of cognitive reserve, also modulates the characteristics of early post-stroke neuroplasticity processes, including the efficiency of reorganization of neural circuits in the ischemic brain area and the flexibility of these neural circuits in recruiting other circuits in adjacent brain areas.[32] In this regard, the brains of ischemic stroke patients with low levels of education have a low capacity to increase the efficiency of their synaptic connectivity disrupted by an ischemic stroke which is necessary to maintain intact cognitive function. Therefore, the patients have a susceptibility to executive dysfunction.[33] Evidence regarding the effect of MTHFR and homocysteine on neuroplasticity is currently insufficient. A study in animal models of stroke has shown that MTHFR deficiency and the resulting hyperhomocysteinemia increase the susceptibility of neurons to ischemic damage. In contrast, supplementation with B vitamins effectively enhances neuroplasticity processes, including through their action on the neurotoxic effects of homocysteine and suppression of DNA demethylation associated with MTHFR deficiency.[34] However, further studies are needed to validate these results.

This study also showed that cigarette smoking and overweight/obesity significantly increased the risk for ischemic stroke-associated executive dysfunction after controlling the identified vascular risk. However, these results are not in line with the previous study results. Pascoe et al.[35] examined the association between the modifiable risk factors and cognitive function among older stroke survivors. The results showed that cigarette smoking and overweight were not associated with cognitive outcomes of the subjects. However, in the absence of stroke, both smoking and being overweight may be related to executive dysfunction.[36,37] The varying results of these studies are mainly due to differences in the population studied, the study design used, and the executive function assessment instruments applied.

This study provides further evidence regarding the contribution of the MTHFR C677T polymorphism as the risk factor for ischemic stroke-associated executive dysfunction. Therefore, these results are valuable to be used as consideration for developing better cognitive rehabilitation intervention strategies, which are specific for executive dysfunction in the studied population or other populations with relatively similar characteristics. However, this study has some limitations. Since this was a cross-sectional study with a relatively small sample size, the results of this study require careful interpretation upon generalization. Further prospective longitudinal studies with larger sample sizes are needed. Some study subjects underwent a head CT scan a few days after the onset of stroke mainly due to the delay of the subjects and their families in recognizing the signs and symptoms of stroke and their lack of access to adequate health care facilities. Since ischemic lesions and cerebral edema evolve over time in the acute and sub-acute phases of ischemic stroke, variations in the timing of the head CT scan may also affect the accuracy in determining infarct size.

CONCLUSION

This study demonstrated that the MTHFR C677T polymorphism (CT/TT), but not hyperhomocysteinemia (≥ 13 µmol/l), was the risk factor for ischemic stroke-associated executive dysfunction. These results become valuable data to consider in developing better cognitive rehabilitation intervention strategies, specifically for executive dysfunction in the population studied or in other populations with relatively similar characteristics.

10 CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

FUNDING

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ETHICAL CONSIDERATION

The ethical approval was from the Ethical Committee for Health Research, Faculty of Medicine, Universitas Hasanuddin (Register number: 1077/UN4.6.4.5.31/PP36/2019).

AUTHOR CONTRIBUTION

Herpan Syafii Harahap (HSH), Muhammad Akbar (MA), Andi Kurnia Bintang (AKB), and Jumraini Tammasse (JT) conceptualized the study. HSH, MA, and Andi Alfian Zainuddin (AAZ) designed the study. HSH drafted the manuscript. All authors analyzed and interpreted the study results and revised the manuscript.

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