The Profile of Angiotensinogenpolymorphism (AGT RS 699) and Working Memory on Hypertension Patients

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Abstract: *Introduction: One of the most common cognitive disorders in hypertensive patients is working memory. Gene polymorphisms associated with hypertension are the ReninAngiotensin System. Angiotensinogen gene known as RAS encoding associated with hypertension is RS 699. Currently, there is not much known about the difference of working memory in hypertensive patients with variant of AGT RS 699 gene compared with work memory score and polymorphism. Objective: To know the difference of working memory score in hypertension patient with AGT RS 699 gene variant by comparing the working memory score of AGT RS 699 gene polymorphism. Method:The sample of 34 people with hypertension in HasanSadikin Hypertension Hospital Polyclinic in Bandung was taken using the sampling technique of sampling. The results were analyzed by comparing the working memory score of AGT RS 699 gene polymorphism through Mini Mental State Examination (MMSE) examination, task list word, recall word list, digit span and omission vigilance. Results:The prevalence of AGT RS 699 polymorphism in hypertension patients was 23.53%. Samples that do not have AGT RS 699 gene polymorphism have result of working memory examination with higher mean value than polymorphism sample. A significant association between AGT RS 699 gene polymorphisms was demonstrated only by checking of the task list (p <0.05). Hypertensive patients with AGT RS 699 polymorphism have a tendency to have a value of the word list task 2.6 times lower than that of non-polymorphism. Conclusion:The risk of working memory on polymorphism of AGT RS 699 gene lower than AGT RS 699 gene which is notpolymorphism.*

Keywords: hypertension, working memory, AGT RS 699.

1. Introduction

Hypertension is one of the risk factors for the occurrence of cardiovascular disease as well as cerebrovascular disease.[1] Earlier reports said the risk of cardiovascular increases when blood pressure above 110/75 mmHg.[1,2] It is estimated that more than 1 billion of the world's population suffers from hypertension and about 7.1 million Death annually.[3]

One of the most common cognitive disorders in hypertensive patients is working memory. Working memory is the process of retaining information when other activities are in progress. This is related to neuroanatomical structures such as the anterior frontal lobes called the prefrontal cortex. This section is responsible for managing working memory, behavior, inhibition of inappropriate behavior, preventing desirability (decreased attention), arranging planning, and structured arrangement.[4,5,6]

Hypertension as a cause of vascular dementia has been widely reported.[7,8,9] Meanwhile, hypertension plays an important role in the onset of Alzheimer's disease.[4-9] Both memory scores and low abstract thought are known to occur before Alzheimer's dementia and diagnosed vascular cognition are diagnosed. Earlier studies also suggested that diurnal hypertension tended to be associated with cognitive impairment depending on age, race and blood pressure classification.[4] On the other hand, a decrease in blood pressure was reported to decrease or reduce the incidence of cognitive impairment or dementia in hypertensive patients.[7,9-12] In other words, it can be concluded that, hypertension is a risk factor for cognitive impairment.

Since genetic pathway has been found in hypertensive cases, there have been many studies of polymorphisms on hypertension that focus on code genes.[13-18] One of the gene polymorphisms associated with this hypertension is the ReninAngiotensin System (RAS).[19,20] Gen Angiotensinogen (AGT) Known as RAS encoding has three variants that are closely related to hypertension, one of which is RS 699 .[19]

Other report has mentioned AGT RS 699 gene polymorphisms associated with an increase in blood pressure.[21] Exposure of small blood vessels in the brain with high blood pressure can cause microvascular disorders, white matter damage, lacunar silent and cortical discontinuation. It has been reported that stroke has a risk of occurring VaD up to 2.8 times.[4] While still debated, M235T (RS 699) also showed hypertensive results in Caucasian, African-Arabian, Japanese and Taiwanan populations.[18] Even other results suggest that polymorphism The RS 699 gene is associated with the progression of atherosclerosis in extracranial as well as intracranial.[22] Based on the above description, the purpose of this study was to determine the difference of working memory in hypertensive patients with AGT RS 699 gene variant by comparing the working memory score of AGT RS 699 gene polymorphism.

2. Material and Method

This research has passed ethical clearence No. 836 / UN6.C1.3.2/ KEPPK/ PN/ 2016 from Medical Faculty of Padjajaran University, Bandung Indonesia. A total of 34

hypertensive subjects from Hypertension Poly-Hasan Sadikin Hospital, Bandung-Indonesia were selected with a sampling technique. Subsequently to the sample was examined hypertension with systolic blood pressure criteria above 140mmHg and diastolic blood pressure above 90 mmHg. Then examined the angiotensinogen polymorphism of RS 699. The research with cross sectional study design was analyzed by comparing the working memory score with AGT RS 699 gene polymorphism based on MMSE examination result, word list task, word list recall, digit span and vigilance omission.

DNA extraction

To obtain the DNA preparation, as much as 300µl of blood was added 900µl 1 × RBC lysis solution in eppendorf tube. Shaken several times, then the solution is kept at least 10 minutes in room temperature. After 10 minutes, the solution is centrifuged at a rate of 13000-16000 RPM for 20sec minutes to obtain leucocyte pellets. The supernatant is removed and repeated again until there is no red blood cell, then homogenized by divortex for about 20 seconds. Then added Cell Lysis Solution as much as 300µl then dihomogenkan by dipipet to melisis cells. An amount of 1.5µl RNA-ase with a concentration of 5mg/ ml was added in the tube and incubated into a waterbath at 37°C for at least 15 minutes. Furthermore, protein protein precipitation solution (100% of 5M ammonium acetate solution is added) then divortex until the solution looks like milk and then centrifuged for 3 minutes. The DNA-containing supernatant was transferred in new eppendorf tubes containing 600µl of isopropanol, then mixed several times until the visible DNA looked like a white thread. After centrifuging for 1 minute the DNA will look like a white precipitate. The supernatant was removed, then 600µl of cold 70% alcohol was added, the tube was reversed for several times to wash the DNA. The DNA pellet is obtained after centrifugation for 5 minutes. The alcohol is then removed and allowed to dry by turning the tube over the tissue paper until the alcohol evaporates. The pellets were then dissolved with 50µl TE buffer pH 8 to obtain the DNA preparation, then the DNA placed in the waterbath for 2 hours then cooled in the refrigerator with a temperature of 4˚C.

Polymerase Chain Reaction

Polymerase Chain Reaction is using kits from Bioline Meridian Bioscience Inc. London, UK. After obtaining DNA, PCR procedure (Bio-Rad Labaoratories, Hercules, CA) is performed. Begin by making a mixture consisting of 21µl H2O, 25µl Mytaq HS Red Mix 2x, 1µl primary F and 1µl primer R, 1µl DNA 100ng / ml. The dosage is for one reaction. All ingredients are inserted in eppendorf tubes in a cold state. The mixture was fed into a 50µl PCR tube and centrifuged for 10s. The next step was to insert PCR tubes into the PCR machine for 2.5 hours programmed for the AGT RS 690 gene genom. The PCR cycle used was 94°C denaturation, 59°C annealing, 72°C elongation. Furthermore the tube is removed and directly used in the electrophoresis stage.The PCR product plus loading dye is then fed into the wells by using a micro pipette. The first well is filled by a marker. Then electrophoresis for 30-60 minutes at 80V voltage. Agarose is seen using UV lights with a wavelength of 300 nm and photos using GelDoc program. An alkaline base of 200 base pairs. In general, there are four stages in the process of mutation detection by DNA sequencing method ie PCR, purification, precipitation and sequencing.

Purification of PCR Products,

Number of 100µl PCR products are inserted into 1.5ml tubes. Then, add buffer 5 times from PCR product volume amount, then in vortex. To bind the DNA, the sample is placed in a DFH column and centrifuged at 14000 RPM for 30 seconds. Supernatant removed. To wash, add 600µl Wash Buffer and then stand for 1 minute and then centrifuged at a speed of 4000 RPM for 30 seconds. The supernatant was discarded, centrifuged for an additional 3 minutes at a rate of 14000 RPM. DFHcolumn put on 1.5 ml tube then added 30µL buffer EB right in the middle of column. Let stand for 2 minutes until completely absorbed and centrifuged for 1 minute. The supernatant is taken and then transferred to a new tube.

Sequencing Cycle

Inserted 4ml mixed BigDye buffer sequencing, dNTPs, ddNTPS dye labeled terminators, 2µl purified PCR products, 1.5µl primers AGT RS 699 13.2mM and ddH20 to 20µl. Included in Gene Amp PCR System 9700-PEBiosystem with denaturation 96° C for 3 minutes, followed by 25 subsequent cycles of 96°C denaturation for 10seconds, anealing at 50°C for 5 seconds and elongation at 60°C for 4 minutes.

Purification of Sequencing Cycle results

On the sequenced product were added 5µl EDTA 125mM and 60µl of absolute ethanol, then divortex and incubated at room temperature for 10 min. Then centrifuged at 5000 RPM for 30 min, the supernatant was removed by the pipette and added 60 µl of 70% ethanol in each tube, then centrifuged for 15 min at a rate of 4000 RPM at 4°C, the supernatant was removed and let the sample dried for 10 minutes Minutes in Speed Vacuum. Samples can be stored at -20°C and protected from light. The fragment is made into the ingredients in the sequencing cycle process ie the attachment of dNTPs (deoxynucleoside triphosphate) fluorescence to the AGT gene.

3. Results

Sequensing Results of RS 699

Sequensing results from 699 CC (No Polymorphism) and 699 CT alleles (heterozygot mutants/Polymorphism) showed color on the base sequence of A green, black G, C blue and T red, in the GA herozyot sequence there are two rising lines simultaneously, it indicates the existence of two heterozygote alleles. The sequensing of RS 699 CC allele (normal) and RS 699 CT allele (Polymorphism) displayed on Figure 1 below:
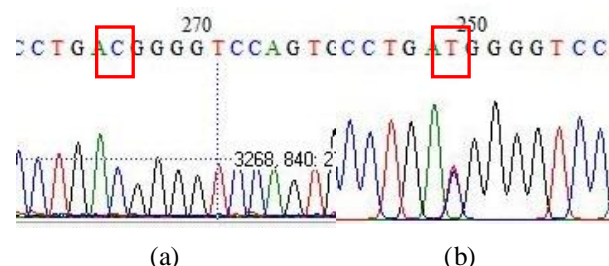

Figure 1: (a) The sequensing of RS 699 CC allele (normal) and (b) RS 699 CT allele (Polymorphism)

Characteristics of patients with hypertension

The results showed that the CT genotype had the same sex characteristics between male and female with age group> 60 years. The CT genotype, education level is dominated by middle level. Employment status is dominated by unemployed and retired. The genotype of CC is dominated by female with age group 46-59 years. The education level is dominated by high level. Employment status is dominated by working. No significant differences were found between the CC (Polymorphism) and CT(No Polymorphism) groups. Characteristics of patients with hypertension displayed on in Table 1 below:

Table 1: Characteristics of patients with hypertension

Characteristics of patients		Genotype				p
		CT		CC		
		n	%	n	%	
Sex	Female	4	50%	22	85%	0.07[a]
	Male	4	50%	4	15%	
Age	<= 45	2	25%	5	19%	0.28[b]
	46-59	2	25%	17	65%	
	>= 60	4	50%	4	15%	
Education	Basic	1	13%	2	8%	0.62[b]
	Middle	4	50%	12	46%	
	High	3	38%	12	46%	
Employment Status	Jobless	3	38%	5	19%	0.11[c]
	Retired	3	38%	5	19%	
	Working	2	25%	16	62%	

Note:
a: Fisher's Exact Test
b: Mann Whitney Test
c: Linear-by-linear association

Distribution of AGT RS 699

Polymorphism of AGT RS 699 has shown that the genotype of single nucleotide polymorphisms (SNP) RS 699 consists of two types namely CT and CC. The CT genotype hereinafter referred to as polymorphism and CC is referred to as non-polymorphism. The prevalence rate of AGT RS 699 polymorphism in the study sample was 23.53%. The Distribution of AGT RS 699 displayed on Table 2.

Table 2: The Distribution of AGT RS 699

SNP	Genotype	Freq. (n)	Percent (%)
RS 699	CT (Polymorphism)	8	23.53%
	CC(No Polymorphism)	26	76.47%
	Total	34	100%

Working memory of AGT RS 699 gene polymorphism

All examinations showed that the group working memory values of AGT RS 699 polymorphisms were lower than those without polymorphism. Different test analysis results show that only the value of word list task has significant difference (p<0.05) compared to other examination. Analysis of the working memory measurement values based on the incidence of AGT RS 699 polymorphism is shown in Table 3 below:

Table 3: Working memory of AGT RS 699 polymorphism

| Exam | CT | | | CC | | | p |
	n	×	SD	n	×	SD	
MMSE	8	28	1	26	29	1	0.19[a]
Word List Task	8	18	1	26	20	3	0.02[b]
Word List Recall	8	6.9	2	26	7.9	2	0.15[a]
Digit Span	8	4.8	1	26	4.9	1	0.93[b]
Vgl. Ommiss	8	0.3	0	26	0.4	1	1.00[b]

Note:
a : T Test 2 independent sample
b : Mann Whitney
CT (Polymorphism)
CC(No Polymorphism)

Prevalence ratio of *Word ListTask* and Polymorphismof of AGT RS 699

Prevalence ratio (PR) was calculated to determine the strength of correlation between AGT RS 699 polymorphism to work memory value. Analysis shows the value of the word list task has a significant difference compared to other examinations. The PR score was 2.6, which means that hypertensive patients with AGT RS 699 polymorphisms have a tendency to have a Word List Task 2.6 times lower than non-polymorphic hypertensive patients in AGT RS 699. A confidence interval of 1.6-4.23 shows the relationship between polymorphism of AGT RS 699 gene with the value of word list task at 5% significance level. Prevalence Ratio Word List Task based on Polymorphism of AGT RS 699 gene can be seen in Table 4 below:

Table 4: Prevalence Ratio Word List Task and Polymorphismof of AGT RS 699

| AGT RS 699 | *Word List Task* | | | | PR | CI |
| | Low | | High | | | |
	n	%	n	%		
CC	8	100	0	0	2.6	1.60-4.23
CT	10	38	16	61.54		
Total	18	53	16	47.06		

Note:
CT (Polymorphism)
CC(No Polymorphism)

4. Discussion

The incidence of AGT RS 699 gene polymorphisms has a risk of working memory values of 2.6 times lower than AGT RS 699 genes that do not experience polymorphism. It has been proven that cognitive impairment is related to the polymorphism of the 699 gene. This disorder can be affected by the cognition of working memory. The existence of a working memory disorder of the working memory of hypertension may disrupt the temporary storage of information that is important for cognitive performance such as reasoning, comprehending, and learning.[23,24,25,26,27]

The existence of the relationship between hypertension, cognition and working memory can cause disruption to the brain. This is in accordance with research reports that mentions, disorders of the brain caused by old hypertension associated with cognitive impairment and adequate blood pressure control during sleep.[4]

Hypertension is associated with disorders that occur in the brain. It is known, there are two types of disorders in the brain associated with it, namely atrophy and cerebral infarction. Earlier research mentions brain atrophy demonstrated by brain imaging found in people with essential hypertension, and single gene defects can affect brain infarction. Other studies have also reported that metabolic disorders that damage the blood vessels both intracerebral and extracerebral can cause atherosclerosis and thromboembolism or insufficiency of hemodynamics.[28]

The study also informed that the genotype of RS 699, both polymorphism and non-polymorphism, showed that both were dominated by aging hypertensive patients. These results are consistent with research reports that show the results of the examination using magnetic resonance imaging for brain imaging of older hypertensive patients [4.22] Some research reports mention one factor is the presence of lesions in white matter. Where, the presence of white matter lesions indicate cardiovascular events such as hypertension, stroke, impaired cognitive function and old age. The further effect is the AGT RS 699 gene polymorphism can cause a decrease in cerebral blood flow which can cause disorders or lesions in white matter [4,6,7,8]

5. Conclusion

Based on the results of the study it can be concluded that the risk of working memory on polymorphism of AGT RS 699 gene lower than AGT RS 699 gene which is notpolymorphism.

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