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The Association among Genotypes of Apoe Gene with Lipid Profile and Internal Carotid Artery Intimal-Media Layer Thickness in Post Ischemic Stroke Patients

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Abstract: Genetic factor has been thought to be a key factor in susceptibility to atherosclerosis, which can progress to ischemic stroke. Apolipoprotein E gene (ApoE) which involved in lipid metabolism, has six genotypes ie ε2/ε2, ε3/ε3, ε4/ε4, ε4/ε3, ε4/ε2, ε3/ε2 and often found in the incidence of atherosclerosis. To evaluate the association among genotypes of ApoE gene with lipid profile and intima-media thickness (IMT) of internal carotid artery, especially atherosclerosis in post-ischemic stroke patients in Dr. Kariadi Hospital Semarang, Indonesia. This was cross sectional study, carried out from January 2011 - March 2011. Sample was taken consecutively and by filling out the questionnaire, blood laboratory tests and examining the genotypes. The internal carotid artery IMT was examined by using carotid duplex ultrasound. The data were analyzed by using appropriate statistical methods. Results revealed that there were significant differences in HDL cholesterol levels and internal carotid artery IMT in ε2/ε3 genotype compared with other genotypes (respectively, p = 0.005 and p = 0.030). There was no association between dyslipidemia and lipid profile abnormalities with ApoE gene genotypes. There was no significant association between ApoE gene genotypes with internal carotid artery atherosclerosis. There was significant differences in LDL cholesterol levels between subjects with and without atherosclerosis (p = 0.044). There were no significant association between various risk factors with IMT, especially internal carotid artery atherosclerosis. There were associations between ApoE gene ε2/ε3 with high levels of HDL cholesterol and lower internal carotid artery IMT in post-ischemic stroke patients. There were no association between ApoE gene genotypes with internal carotid artery atherosclerosis. There was association between LDL cholesterol levels with internal carotid artery atherosclerosis.

Key words: ApoE Gene · Internal Carotid Artery IMT · Atherosclerosis · Lipid · Ischemic Stroke

INTRODUCTION

Intracranial atherosclerosis is one of the important causes of ischemic stroke [1-3]. About 14-40% of ischemic stroke caused by atherosclerosis [4]. Epidemiology data show that Asia, Africa and Hispanic races have high risk of intracranial atherosclerosis. Differences in genetic background, diet, levels of physical activity, age and sex structure all influence the prevalence of both metabolic syndrome and its components [5]. Genetic factor are also assumed to have a relationship with a certain subtype stroke [6-8]. This can be prevented by finding out that an individual's gene that susceptible to atherosclerosis and stroke [7].

Many researchers believe that the impact of genetic in medicine will revolutionize the concept of human health [9] and they have been conducted to find out gene candidates involved in stroke. Mostly focused on the genes that play the roles in atherosclerosis process through the lipid metabolism pathology [10]. One of them is apolippoprotein E (ApoE) [7]. Apo E gene is located in 19q13.2 chromosome which has three most common alleles. They are epsilon (ε)2, (ε) 3 and (ε) 4, coding three ApoE isoform that is E2, E3, E4. The three alleles resulted six genotype ε2/ε2,ε3/ε3,ε4/ε4,ε4/ε3,ε4/ε2,ε2/ε3 that coding Apo E. Allele ε3 is the most common gene found in all population. So the ApoE3 result is considered as the normal protein. Meanwhile, the ApoE2 and ApoE4 is

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considered as the variant [11-13]. ApoE play a role as liquid uptake mediator by the tissue and primary liquid transport mediator in the brain. It is related with the lipoprotein serum level [7, 14, 15]. Lipid from food will enter the blood circulation in a form of chylomicron particle which is rich of triglycerides. After lipolysis process completed, there are cholesterol rich residue and it has aterogenic potention. The cleaning process of the residue depends on the role of ApoE related with receptor of low density lipoprotein (LDDR) and LDLR protein binder (LRP). Apo E3 and ApoE4 have equal binding ability, but ApoE2 has only 2% binding ability to LDLR and 40% to the LRP. It caused the postponed of both chylomicron and very low density lipoprotein (VLDL) residue catabolism and lead to the accumulation of the particles in the plasma. The existence of lipoprotein (VLDL) residue mediated by ApoE to low density lipoprotein (LDL) caused accumulation VLDL residue in the plasma and the low result of LDL. The low transfer of rich cholesterol residue particle to the liver and the decrease of LDL formation level caused upregulation of LDL receptor. The changes mediated by ApoE on the lipoprotein metabolism results concentration of residue particle plasma containing high cholesterol, triglycerides and the low LDL particle level with rich cholesterol. Oxidative modification of low density lipoprotein cholesterol (LDL-C) as a causative agent in atherogenesis leading to coronary heart disease has recently been documented [16]. We have less research about lipoprotein metabolism by ApoE 4, researches mostly related to ApoE2. ApoE2 is slightly related with VLDL and LDL. It is much more related with high density lipoprotein (HDL) compared with ApoE 3, while ApoE4 is much more related with VLDL and LDL. It is slightly related to HDL [11]. HDL is an antiatherogenic molecule and is the only negative risk factor for cardiovascular disease [17]. The difference on intake postprandial lipoprotein particle result difference in the regulation of LDL in liver then later will cause the relation of genotype difference with total cholesterol level and LDL cholesterol [11].

ApoE gene polymorphism is predicted to be responsible for 4%-8% of the variant of total cholesterol level and LDL cholesterol on Caucasian population.16 Interaction between ApoE genotype and the environment like age, smoking habit and obesity participate in the emergence of the atherosclerosis and cerebrovascular disease [18-21].

Many researches have proven a relationship between ApoE gene and plasma lipid level. Allele $\varepsilon 4$ is related with the increasing of LDL cholesterol which takes roles in atherosclerosis process. Allele $\varepsilon 2$ is related with the decreasing of LDL level, so it has atherosclerosis characteristic [7, 15, 22-27].

Genetic influence is found mainly on ischemic stroke subtype caused by atherosclerosis of great vessel [6, 23]. This, become a basic to develop researches about stroke and carotid artery [22, 23, 28]. Intima Media Thickness (IMT) of artery carotid measured by using ultrasound shows the existence of atherosclerosis even in the early stadium, so it could be used as stroke predictor. Some researches show that ApoE gene has an effect on IMT of artery carotid [22, 25, 28, 29], but the other research failed to prove it [24]. ApoE was also found to have relationship with the forming of carotid plaque [24, 28]. A metaanalyse studies conducted on 22 publications of research found relationship between ApoE and IMT of artery carotid, resulted in the increasing of tendency of atherotrombotic stroke incidence [29].

Many researches about ApoE gene allele genotype on different races shows different allele frequency with the influence on the blood lipid profile and with different atherosclerosis as well [11]. There is no data related with lipid profile and IMT of great artery, especially atherosclerosis on the post-ischemic stroke patients in Semarang, Indonesia. This study is aimed to find data about ApoE Allele genotype and its association with lipid profile and IMT of internal carotid artery on the post-ischemic stroke patients in Semarang.

MATERIALS AND METHODS

The research was conducted in cross sectional method by consecutive sampling of outpatient post ischemic stroke who had their check-up examination in the neurology clinic of Dr Kariadi Hospital Semarang, from January 2011 to March 2011. The inclusion criteria are ischemic stroke verified by brain CT Scan, one month to 5 years onset, having medication treatment for stroke risk factor and has signed the Informed Consent. The exclusion criteria are patients with febrile seizures history, tonic-clonic seizures with febrile seizures history, patients with familial hemiplegic migraine, episodic ataxia and spino cerebral ataxia.

Demographic data including age, sex, education level, job and medical record were collected using structured questionnaire. Then, general physical and neurological examination was conducted. Moreover, the laboratory testing for blood glucose profile (blood glucose during fasting and 2 hours postprandial), total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride were done.

Table 1: Shows the general characteristic of the subjects.

		Aterosclerosis (+) n = 10 (25,6%)		Aterosclerosis (-) n = 29 (74,4%)		
		N	%	n	%	
Sex	Male	7	23,3	23	76,7	0,669
	Female	3	33,3	6	66,7	
Age	> 55	10	33,3	20	66,7	0,079
	≤ 55	0	0,0	9	100	
Smoking history	Smoker	3	21,4	11	78,6	0,721
	Not smoker	7	28,0	18	72,0	
Hypertension history	Yes	9	28,1	23	71,9	0,653
	No	1	14,3	6	85,7	
Diabetic history	Yes	2	16,7	10	83,3	0,693
	No	8	29,6	19	70,4	
Heart disease history	Yes	2	22,2	7	77,8	1,000
	No	8	26,7	22	73,3	
Dyslipidemia history	Yes	8	28,6	20	71,4	0,693
	No	2	18,2	9	81,8	
Obese	Yes	3	27,3	8	72,7	1,000
	No	7	25,0	21	75,0	
Blood Pressure Abnormality	Yes	9	23,7	29	76,3	0,256
	No	1	100,0	0	0,0	
Blood glucose Abnormality	Yes	2	14,3	12	85,7	0,279
	No	8	32,0	17	68,0	
Lipid profile Abnormality	Ya	10	27,0	27	73,0	1,000
	No	0	0,0	2	100,0	
ApoE Genotype	ε2/ε2	0	0,0	2	100	0,239
	ε2/ε3	0	0,0	5	100	
	ε3/ε3	4	21,1	15	78,9	
	ε4/ε2	1	50,0	1	50,0	
	ε4/ε3	5	45,5	6	54,5	

The genotype of ApoE was examined in molecular biology laboratory, medical faculty of Gajah Mada University. DNA was extracted from 5 ml vein blood. Then Polymerase Chain Raction (PCR) was performed by using primer F5TCCAAGGAGCTGCAGGCGGCGCA and R5'GCCCGGCCTGGTACACTGCCA.Restrictionfragment length polymorphism (RFLP) method with AF3 and Haell enzyme was conducted to find out the subject of ApoE gene genotype [30, 31]. Internal carotid (IMT) was measured using USG duplex by a radiographer. The measurement was located in 3 cm proximal and 1 cm distal of the bifurcation right–left-carotid. We collected the data of the thickest right left carotid IMT. Atherosclerosis diagnosis was made if the thickness > 0,9 mm.

Statistical analyses was using SPSS15 software in univariant analysis to see the whole description of the research data bivariant analysis to test the relation between ApoE gen genotype and internal carotid artery atherosclerosis were conducted using Chi-square with 95% of the trust level or Fisher's Exact test. The difference of lipid level and IMT in different genotype group was tested using unpaired t-test or mann-whitney test correlation between numeric /correlation test or spearman-rho test. The association among variable was considered significant if $p<0.05$.

This study has been approved by the hospital ethics research committee and Faculty of medicine Diponegoro University.

Research Result: This research involved 39 sample fulfilled the inclusion and exclusion criteria:

General Characteristic: This research found 10 subject (25,6%) had atherosclerosis (IMT 70,9mm). Most of the subject were male and over 55 years. Subjects under 55 years had no incidence of the atherosclerosis. Subject with atherosclerosis were found out to have 21,4% smoking history, 28,15% hypertension history, 16,7% DM history and 22,2% heart attack history. Physical and laboratory examination found that on the subject with atherosclerosis, 3 person (27,3%) had obesity, 9% (23,7%) hypertension, 2 person (14,3%) with abnormal blood glucose profile and 10 person (27,0%) with abnormal lipid profile.

Tabel 2: Physical Examination and laboratory characteristics in subjects with aterosclerosis and without aterosclerosis.

		Aterosclerosis (+)	Aterosclerosis (-)	
	n = 39			
	average±SB	n = 10(25,6%) Rerata±SB	n = 29 (74,4%) Rerata±SB	*p*
Systolic Blood pressure (mmHg)	141.28 ±17,50	141,00 ±19,69	141.38±17,06	0,792*
Diastolic Blood Pressure (mmHg)	87,67 ±11,50	84,40 ±16,68	88,79±9,23	0,465*
Fasting Blood Glucose (mg/dl)	109.87 ±36,42	105,80 ±24,34	111,28 ±40,02	0,760*
2 hours Post Prandial Blood glucose (mg/dl)	162,26±54,49	164,00±63,48	161,66±52,28	0,995**
Trigliserida (mg/dl)	118,38±43,73	117,10±33,88	118,83±47,17	0,867**
Total Cholesterol (mg/dl)	184.26±35,50	197,90±38,87	179,55±33,70	0,162**
LDL Cholesterol (mg/dl)	113,89±25,85	128,80±32,77	108,75±21,33	0,044**
HDL Cholesterol (mg/dl)	41.41±7.40	42,20±5,67	41,14±7,98	0,701**
HbA1C (%)	5.59±0,93	5,64±0,66	5,57±1.02	0,838**
BMI (kg/m2)	24,21±3,62	25,39±4,66	23,79±3,18	0,237**
IMT (mm)	0,83±0,24	1,16±0,18	0,72±0,13	

Tabel 3: Bivariat analysis results between risk factors variable ang internal carotid artery IMT

	Intimal-media layer thickness (IMT) (mm)	
Variables	Coefficient corelation *r*	*p*
Age	0,205 ¥	0,210
Systolic blood pressure	0,059 $	0,723
Diastolic blood pressure	-0,130 $	0,429
BMI	0,242 ¥	0,136
Fasting blood glucose	-0,033 $	0,843
2PP blood glucose	-0,095 ¥	0,563
HbA1C	-0,130 ¥	0,429
Total cholesterol	0,026 ¥	0,875
Trigliserid	-0,144 ¥	0,381
LDL Cholesterol	0,034 ¥	0,836
HDL Cholesterol	-0,017 ¥	0,917
Sex		0,336*
Smoking history		0,524*
- Yes		
- No		
BP Abnormality		0,300*
- Yes		
- No		
Blood Glucose Abnormality		0,224*
- Yes		
- No		
Lipid profile Abnormality		0,723*
- Yes		
- No		
Obesity		0,297*
- Yes		
- No		

ε3/3 was the most frequent ApoE genotype 948,7%. No subject with ε2/2 and ε2/3 genotype had atherosclerosis. Whereas, 21,4% subject with ε3/3 genotype had atherosclerosis. In other hand, atherosclerosis was found on ε4/2 and ε4/3 genotype with 50% and 45,5%. There was no significant relationship between general characteristic of the subject, risk factor history, ApoE genotype and atherosclerosis.

Characteristics of Physical Examination, Laboratory Test and USG Duplex: The average of the systolic pressure, diastolic pressure, fasting blood glucose level, 2 hours PP blood glucose level, total cholesterol level and BMI, triglycerides level was found higher HDL cholesterol level was found lower in the subject without atherosclerosis than those with atherosclerosis. Nevertheless, there was no significant difference (Table 2)

The average of LDL level was higher on the subject with atherosclerosis. It had significant difference compared with the subject without atherosclerosis (p=0.044). However, the data on Table 3 shows no significant correlation between risk factor of atherosclerosis and internal carotid IMT.

The Relationship Between Genotype and Lipid Profile with IMT: The average of total cholesterol level, LDL, Cholesterol and the highest triglycerides was found in the ε3/ε4 genotype group. Also, the average of the thickest carotid IMT was found on it. However, there was no significant association between ε4/ε3 genotype and the total cholesterol level, HDL, cholesterol, LDL cholesterol and IMT.

The average of the lowest triglycerides level and the highest HDL was found in ε2/ε3 genotype and the lowest LDL cholesterol in ε4/ε2 genotype.

There was significant relation between ε2/ε3 genotype and HDL cholesterol level (p= 0.005) and IMT (p=0.03), where in this genotype group, the highest average in HDL and the lowest is internal carotid IMT.

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Tabel 4: Bivariat analyzed results between lipid profile variable and internal carotid artery IMT in vary genotypes groups

	Total Cholesterol		Trigliserid		LDL Cholesterol		HDL Cholesterol		IMT	
Genotype	average±SB	*p*	average±SB	*p*	average±SB	*p*	average±SB	*p*	average±SB	*p*
ε2/ε2	150±32,53	0,164	107,5±28,99	0,842	98,50±37,48	0,292	31,50±0,71	0,050	0,90±0,00	0,551
ε2/ε3	196,40±34,17	0,420	123,2±50,03	0,880	105,80±17,31	0,508	49,80±7,73	0,005	0,64±0,13	0,030
ε3/ε3	183,84±31,34	0,944	109,89±44,522	0,217	115,25±26,34	0,719	41,16±6,56	0,839	0,80±0,20	0,524
ε4/ε2	209,50±85,56	0,308	116,00±24,04	0,897	98,00±7,071	0,398	41,00±4,24	0,937	1,15±0,35	0,075
ε4/ε3	181,09±35,64	0,732	133,27±46,23	0,196	120,90±28,91	0,306	39,91±6,88	0,434	0,91±0,28	0,258

DISCUSSION

This research had 24,6% subjects with atherosclerosis which is in line with the previous research that found 14,40% ischemic stroke caused by atherosclerosis [4].

Atherosclerosis is one of the disease that can be caused by various factors like age, sex, smoking history, hypertension history, DM history, dyslipidemia history, obesity, blood pressure, blood glucose profile and blood lipid [30]. In this research there was no significant difference between who subjects have got atherosclerosis and those who do not (Table 1 and 2). Bivariate analysis did not show significant correlation between history and risk factor parameter with IMT (Table 3). It is probably because the risk factor of the research was relatively controlled like what is shown in table 2. Moreover, the subjects with smoking history had already stop after the stroke attack. The controlling of the risk factor probably could modify atherosclerosis progress on the post ischemic stroke patients. Other possibility is that the time period the subjects suffer from the risk factor was not examined as the research variable.

The most common genotype are allele $\varepsilon3/\varepsilon3$ followed by allele $\varepsilon4/\varepsilon3$, allele $\varepsilon2/\varepsilon3$ and the last was allele $\varepsilon2/\varepsilon2$ and $\varepsilon4/\varepsilon2$ (Table 1). This research on the Asian population and the Caucasian got allele $\varepsilon3/\varepsilon3$, as the most ApoE genotype which is considered as the wild type allele. The most ApoE gene polymorphism in China is allele $\varepsilon4/\varepsilon4$ and the last is allele $\varepsilon2/\varepsilon$ which is the same as what is found in this research [31].

There was no subject with atherosclerosis on genotype $\varepsilon2/\varepsilon2$ and $\varepsilon2/\varepsilon3$, whereas almost half of the subject with genotype $\varepsilon4/\varepsilon3$ had atherosclerosis. The result agree with the previous research which found genotype $\varepsilon3$ related with atherogenic effect, while $\varepsilon2$ was atheroprotective characteristic [24, 28, 32].

Atherosclerosis process on ApoE gene genotype $\varepsilon4$ might be explained through the high average of total cholesterol level, LDL cholesterol and blood triglycerides compared with another genotype group although it is not statistically significant. The result confirms the previous research which found the triglycerides increase, total cholesterol and IMT on the subject with genotype $\varepsilon2\varepsilon$ had the average of total cholesterol total, LDL cholesterol, the lowest triglycerides and the highest HDL. In addition genotype $\varepsilon2\varepsilon3$ had an association with HDL cholesterol level and the average carotid IMT significantly (Table 4). Atheroprotective $\varepsilon2$ effects predicted to go on by the work of $\varepsilon2$ apolipoprotein which more related with HDL if it compared with other genotype [11]. HDL is atheroproctective as it took and carried cholesterol from peripheral tissue mainly to the liver for being degraded. Besides HDL has antioxidant effect, anti-inflammation, vasodilation and antithrombotic [33].

This research found no significant association between gene allele ApoE $\varepsilon2\varepsilon2$ $\varepsilon3\varepsilon3$ $\varepsilon4\varepsilon3$ $\varepsilon4\varepsilon2$ with internal carotid artery IMT. This result was not compatible with the main result of the previous research [7, 8, 25, 28, 29, 31, 34]. Probably the genetic towards cerebral and stroke atherosclerosis might be located in the more specific part of the ApoE gene. The research by Abbout et al found that promoter of polimorphism of ApoE gene (single nucleotide) was the ischemic stroke predictor [23]. The other possibilities are the ApoE allele genotype has no relation with IMT,but it is more related with carotid plaque which might be the advanced stage of multiple sclerosis that there was specific genetic factor involved in carotid plaque formation but it is not involved in the thickness process of intima media tunica [10, 22, 28]. The other reason is that IMT could not differentiate the cause of the artery wall thickness whether all of the thickening athreosclerosis thickening but the thickness could be the cause of adaptive respond towards the stress shear changing [28]. The inconsistae result on the various researches might be caused by the different heterogen method of IMT measurement [28]. Genetic effect may be different between subtype stroke of great and small vessels [6, 7]. This research found the association between genotype $\varepsilon2\varepsilon3$ and internal carotid artery IMT. It failed to prove the association between genotype and internal carotid artery atherosclerosis. It is probably because the atherosclerosis criteria that is IMT >0,9 mm, was found in he research on the caucasian race

and not on the Indonesian. IMT criteria for the atherosclerosis in indonesian might be different from the criteria in Caucasian and there is not yet any research in indonesia about IMT that could be considered as atherosclerosis. Besides, atherosclerosis incidence is a complicated process with multifactorial ethiology, so probably it is not only one gene who is responsible to the case of atherosclerosis, but is the interaction between two or more genes, like what Myllykangas et studied. They found the interaction between ApoE gene and lipase lipoprotein gene (LPL) towards atherosclerosis carotid [35]. Additional probability is that ApoE level in the serum coded by the gene take role in the atherosclerosis process [14, 27, 36].

Research limitations were excluding the variable of time period the patient suffer from risk factor such as smoking, DM, Hypertension, Dyslipidemia and obesity. Exercise variable that could influence atherosclerosis was not measured as well. This research was held at Dr. Kariadi hospital with small number of sample from neurology outpatient clinic. It could not describe the real population of ischemic stroke yet. Moreover, most of the subjects have been controlled by the regular medicine treatment.

CONCLUSION

There was an association between º2/º3 ApoE gene genotype with high HDL-Cholesterol level and the low internal carotid artery IMT on the post ischemic stroke patient.

There was no significant association between ApoE gene genotype with history of Dislipidemia, abnormality of blood lipid profile and internal carotid artery atherosclerosis.

Suggestion: ApoE gene examination is necessary to find out the susceptibility to Atherosclerosis as both preventive and therapeutic to atherosclerosis as well as if there is dislipidemia and the resistance to the therapy.

Further research is needed to find out the relationship among genotype of ApoE gene with ApoE serum and their association with IMT and atherosclerosis. Besides, it is necesary to carry out the research to the sequence of ApoE gene to find out the single nucleotype polymorphism which probably play role in the susceptibility to Atherosclerosis and ApoE gene interaction with the other gene and with the environtment factor affecting atherosclerosis process.

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