Polymorphism of apolipoprotein C3 (APOC3) gene in children with HIV infection at Dr. Soetomo Hospital, Surabaya, Indonesia

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Abstract

Background: Genetic factors can affect the therapeutic response to HIV infection. Apolipoprotein C3 (APOC3) polymorphism is related to metabolic disorders and hypersensitivities and affect tolerability that impacts adherence to highly active antiretroviral therapy (HAART).

Objective: To analyse the polymorphism of APOC3 gene in children with HIV infection in Dr. Soetomo Hospital, Surabaya, East Java, Indonesia.

Method: A descriptive observational study was conducted in Dr. Soetomo Hospital Surabaya from September 2019 to January 2020 in 2-13 year old children with HIV infection. APOC3 was determined by restriction fragment length polymorphism analysis. Statistical analysis was done using Fisher test. p <0.05 was taken as significant.

Results: Of the 24 patients, 11 (45.8%) were boys and 13 (54.6%) were girls. Mean age was 7.5 ± 0.59 years. Twenty three (95.8%) mothers with HIV-infected children also had positive HIV status. Sixteen (66.7%) children had a duration of therapy less than 5 years. Mean age of child at diagnosis was 4.2 ± 0.56 years. Mean CD4 absolute value was 769.61 ± 439 cells /cu mm, and the mean CD4% value was 15.49 ± 6.4%. Seven (29.2%) patients had the wild type APOC3 gene, and 17 (70.8%) patients had the homozygous mutant APOC3 gene. There was no significant relationship between sex (p=0.059), duration of therapy (p=0.218) or history of changing therapy (p=0.708) with polymorphism of APOC3 gene.

Conclusions: At Dr. Soetomo Hospital, presence of APOC3 gene polymorphism in children with HIV infection was 70.8%.

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(Key words: HIV Infection, APOC3, genetic)

Introduction

The genetic factors of the host can influence the response to HIV infection. Genetic polymorphisms related to HIV infection influence lipid metabolism[1]. Polymorphism of the Apolipoprotein C3 (APOC3) gene is associated with decreasing high density lipoprotein (HDL) and non-HDL in adult patients, and affect the total cholesterol levels in children. The polymorphisms of APOC3 gene will be associated with metabolic disorders and fat metabolism in the body. The presence of metabolic disorders is associated with high levels of lipids in the blood associated with hypersensitivity reactions to drugs so that it can lead to therapeutic failure from anti-retroviral therapy (ART)[2].

APOC3 gene encodes protein components of Triglyceride Rich Lipoprotein (TRL) including very low density lipoprotein (VLDL), HDL and chylomicrons. The APOC3 gene plays an important role in the metabolism of the lipoprotein that initiates the secretion of VLDL, inhibits the activity of the lipoprotein lipase enzyme, and inhibits remnant TRL catabolism[3]. The APOC3 gene shows a significant effect as a predictor of total cholesterol. Genetic determination with APOC3 has a large impact in the selection of therapeutic regimens and predicts the side effects of highly active antiretroviral therapy (HAART) drugs[2].

Globally in 2014, of 2.6 million children aged <15 years infected with HIV, 88% lived in sub-Saharan Africa[4]. From 2010 until 2017 in Indonesia there were 3055 children infected with HIV[5]. However, in Indonesia there is currently no research on genetic factors for host HIV infection in children.

Objectives

To analyse the polymorphism of APOC3 gene in children with HIV infection in Dr. Soetomo Hospital, Surabaya, East Java, Indonesia.

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Method

Study population comprised a cross section of 2-13 year old children with HIV recruited from Infection Outpatient-Clinic and hospitalized patients in Dr. Soetomo Hospital.

Amplification of the APOC3 promoter region: Genomic DNA was amplified by polymerase chain reaction (PCR) from 24 participants. The following primers were used to amplify a 238 bp gene fragment: APOC3 5'-GGATTGAAA CCCAGAGATGGAGGTG-3' (sense) and 5'-TTCACACTGGAATTTCAGGCC-3' (antisense). The PCR reaction mixture comprised a final concentration of 4µM of each primer, 200µM dNTP 4U FastStart Taq polymerase (Roche Diagnostics, Mannheim, Germany), 5µL 10× PCR buffer (MgCl2, 25mM), 5µL of DNA template and nuclease free water in a 50µL total reaction volume. Cycling conditions comprised an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 2 min, annealing at 55°C for 1 min, elongation at 72°C for 2 min and a final elongation at 72°C for 10 min. The resulting PCR products were resolved by electrophoresis on a 1% ethidium bromide-stained agarose gel (Invitrogen, Carlsbad, CA, USA).

Genotyping of APOC3 promoter region: Amplified DNA was purified with a Qiagen DNA purification kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Genotypes of APOC3 were determined by RFLP. Restriction enzymes, FokI (restriction site: 5'-GGATG(N)9^-3', Fermentas, Vilnius, Lithuania) were used for restriction sites. The digestion mixture was incubated at 37°C for 30 min. Digestion products were resolved on 2% ethidium bromide-stained agarose gels (Invitrogen, Carlsbad, CA, USA).

Ethical issues: Study protocol received approval from the Dr. Soetomo Hospital Research Ethics Committee (No. 1447/EPK/VIII/2019) Study was carried out after obtaining written consent from participants or their parents after explaining to them in detail the study demographics, sample collection and sequencing DNA extraction procedures

Statistical analysis was done using Fisher exact test with a p value <0.05 being considered significant. Data was processed with SPSS software.

Results

Subject characteristics of the 24 participants are shown in Table 1. The youngest was 2 years old and the oldest 13 years old. The 3 deaths were due to opportunistic infection and septicaemia.

Table 1: ***Subject characteristics (n=24)***

Characteristic	Result
Sex - number (%)	
Boys	11 (45.8)
Girls	13 (54.2)
Mean age in years	7.5 ± 0.59
Mean age in years when HIV was diagnosed	4.2 ± 0.56
Duration of therapy - number (%)	
< 5 years	16 (66.7)
≥ 5 years	08 (33.3)
Change ARV - number (%)	
No	23 (95.8)
Yes	01 (04.2)
Residence - number (%)	
Surabaya	20 (83.3)
Other than Surabaya	04 (16.7)
HIV status of mother - number (%)	
Positive	23 (95.8)
Negative	01 (04.2)
HIV status of father - number (%)	
Positive	19 (79.2)
Negative	05 (20.8)
Delivery process - number (%)	
Spontaneous	22 (91.7)
Caesarean section	02 (08.3)
Comorbid illness - number (%)	
None	13 (54.2)
Tuberculosis	04 (16.7)
Cardiomyopathy	01 (04.2)
Pneumonia	01 (04.2)
Thalassaemia	02 (08.3)
Cerebral palsy	01 (04.2)
Septicaemia	02 (08.3)
Outpatient - *number (%)*	14 (58.3)
Hospitalized - *number (%)*	10 (41.7)
Outcome - number (%)	
Survival	21 (87.5)
Death	03 (12.5)

From this study amplification was carried out on all research subjects, then continued with RFLP. The genotype for the APOC3 promoter gene of 238 bp was cut with the FokI restriction enzyme. The results of the study appear in Figure 1

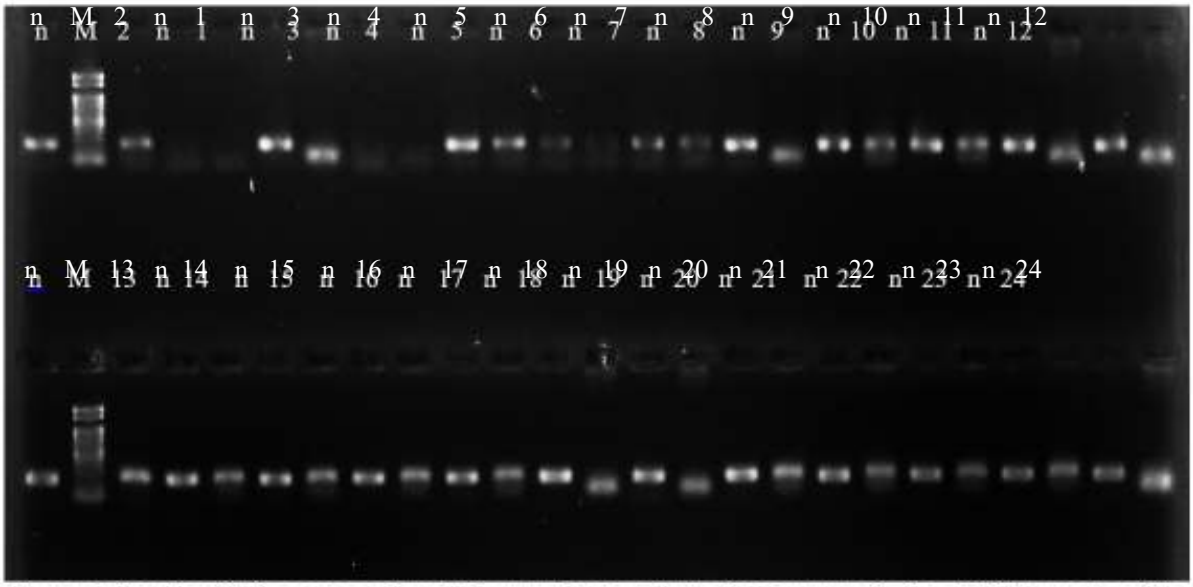

Figure 1: ***Results of 2% agarose gel restriction products with the FokI enzyme for the APOC3 genotype. The restriction pattern with FokI enzymes is as follows: lane M, 100bp as molecular marker; lane is a variant of the CC genotype (no cut), whereas lane is a TT / wild type variant (cut with enzymes), a single band indicates no place of restriction.***

From the research results we obtained various variants of the APOC3 gene, namely TT (Homozygous, wild type) and CC (Homozygous, mutant). From 24 samples, the prevalence of wild type was 7 (29.2%) patients, while the prevalence of homozygous mutations was 17 (70.8%) patients. The analysis of APOC3 in children with HIV infection is shown in Table 2.

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Table 2: ***Analysis of APOC3 in children with HIV infection***

Variables	APOC3		*p**
	***Wild Type* - Number**	**Homozygote - Number**	
Male	1	10	0.059
Female	6	7	
Therapy < 5 years	6	10	0.218
Therapy ≥ 5 years	1	7	
No change ART	7	16	0.708
Change ART	0	1	
Outpatient	3	11	0.269
Hospitalized	4	6	

**p* <0.05 significant, *Fisher exact test*

Discussion

The gene polymorphisms will influence HIV infection in children. One of them is the APOC3 gene which is related to fat metabolism and distribution. The prevalence of homozygous mutations was 17 (70.8%) patients. Wild type alleles show that the genes are in good condition and have a good function so that they are related to the low possibility of metabolic disorders. This study showed the low frequency of wild type alleles in HIV infected children in Surabaya. The low frequencies of wild type alleles of APOC3 in Caucasian and Hispanic races are predominant factors in the occurrence of metabolic disorders characterized by high levels of triglycerides. The presence of metabolic disorders is associated with high blood lipid levels associated with hypersensitivity reactions to drugs and leads to the failure of therapy from ART[4]. Thus, genetic determination of the APOC3 gene has a large impact in the selection of therapeutic regimens and predicts the side effects of HAART drugs[2].

In this study there was no gender relationship with the polymorphism of the APOC3 gene. The mechanism by which sex hormones influence the APOC3 gene on lipid metabolism is still unclear. According to a meta-analysis study by Cheng S, *et al*[6], triglyceride concentrations in women carrying variants of the APOC3 SstI allele were higher than in men. There was a relationship between the polymorphism of the APOC3 gene and triglyceride levels, but there was no gender difference in the expression of the APOC3 gene[6]. According to the

study by Ruel TD, *et al*[7], the existence of gender differences affects the immunological status of HIV infected children. This is due to the immunomodulatory effects of female sex hormones that influence the expression of CCR5 via CD4 and produce several cytokines. In addition, female sex hormones and fluctuations during the ovulation cycle will modulate innate and adaptive immune responses. Then it will affect the replication rate of the HIV virus[7].

The polymorphisms on the APOC3 gene is associated with metabolic disorders and fat metabolism in the body. The presence of metabolic disorders is related to the high lipid levels in the blood associated with hypersensitivity reactions to drugs so that it can lead to therapeutic failure from ART[2]. Genetic determination of the APOC3 gene has a large impact in the selection of therapeutic regimens and predicts the failure of HAART drugs in children with HIV infection in Africa[8,9]. Highly active ART brings about significant improvement, improving the quality of life of HIV children[10]. The influence of race / ethnicity, APOC3 genotype, and exposure to protease inhibitors are significant predictors of HDL cholesterol levels in HIV infection. The results of the study showed low triglyceride levels and high HDL cholesterol levels in black/non-Hispanic races compared to Caucasian and Hispanic races[11].

The presence of APOC3 gene polymorphisms will cause overexpression of the APOC3 gene, thus increasing the synthesis of plasma triglycerides and the accumulation of VLDL and insulin secretion through the mechanism of calcium channel intervention in pancreatic beta cells, excess insulin synthesis causing insulin resistance[12]. A study by Tarr PE, *et al*[13] examining APOE and APOC3 genotyping in adults with HIV infection in Switzerland showed that people with HIV infection who received ritonavir therapy had a risk of developing hypertriglyceridaemia. The study showed that variant alleles of APOE and APOC3 contributed to the lipid profile of HIV patients. The interaction between genotypes and ARVs will cause hyperlipidaemia. This study showed the presence of polymorphisms associated with hyperlipidaemia in the European continent[13].

The mechanism of metabolic changes associated with the administration of antiretroviral drugs is still being debated. Protease inhibitors can initiate adipocyte differentiation apoptosis. In addition, protease inhibitors inhibit pre-adipocyte differentiation even if only in vitro. The existence of these mechanisms contribute to lipoatrophy and the release of fatty acids which then reach the liver. This causes the liver to synthesize triglycerides and more VLDL[14]. The use of protease inhibitors will disrupt the normal regulation of the APOC3 gene, causing over-expression of the APOC3 gene and affecting the transcription process of the APOC3 gene. Over-expression of the APOC3 gene will directly affect insulin production in pancreatic beta cells. Insulin will inhibit the work of the APOC3 gene so that the down regulation of APOC3 gene expression is down. The APOC3 gene polymorphisms will disrupt the down regulation process[11].

The presence of polymorphisms in the APOC3 promoter gene will influence the regulation of APOC3 expression. Evaluation of allele distribution of promoter variations in certain population races is very important to know the existence of variations in APOC3 activity. Studies in Kuwait show that the high frequency of APOC3 polymorphism is 49.5%. This figure is higher than in the Caucasian population at 42%. Analyzing the frequency of polymorphisms in different ethnic populations showed a high frequency in African-American ethnicity (71.2%), Hispanic (38.9%) and European (36.6%). The different frequency of alleles in different ethnic groups shows that ethnic differences affect the expression of APOC3 genes that are different in each ethnicity[15].

A study by Van Den Hof M, *et al*[16] in the Netherlands, shows that differences in APOC genotype contribute to differences in LDL levels and also lipoprotein levels in children with perinatal HIV infection compared to controls[16]. The presence of APOC3 gene polymorphisms will affect serum triglyceride levels in HIV patient populations in Thailand but unfortunately research on the Asian Continent is only limited to adults[17].

The strength of this study is that this is the first study in Indonesia and even in Southeast Asia which examined the presence of gene polymorphisms from host factors for HIV infection in children. The weakness of this study is the small number of research samples so that further research needs to be done with a larger sample size.

Conclusions

The presence of APOC3 gene polymorphism in children with HIV infection is 70.8%.

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