Apolipoprotein A5 (APOA5) -1131T>C polymorphism and dyslipidemia in Thai HIV/TB co-infected patients receiving efavirenz-based antiretroviral therapy

Thatchapon Boontoog[1,2], Weerawat Manosuthi[3], Pornpen Srisawasdi[4], Apichaya Puangpetch[1,2], Poonpilas Hongmanee[5], Chonlaphat Sukasem[1,2]

[1]Division of Pharmacogenomics and Personalized Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[2]Laboratory for Pharmacogenomics, Somdech Phra Debaratana Medical Center (SDMC), Ramathibodi Hospital, Bangkok, Thailand

[3]Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand

[4]Division of Clinical Chemistry, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[5]Division of Microbiology, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

ABSTRACT

Data on the association of *apolipoprotein A5* (*APOA5*) polymorphism and dyslipidemia in Thai HIV and tuberculosis (HIV/TB) co-infected patients receiving efavirenz-based antiretroviral therapy (ART) are very limited. The aim of this study was to investigate the association between *APOA5* -1131T>C polymorphism and dyslipidemia among Thai HIV/TB co-infected patients receiving efavirenz-based antiretroviral therapy. One hundred and twenty-one of antiretroviral-naïve HIV/TB co-infected patients were enrolled. Clinical and laboratory data including lipid profile were collected at baseline and 48 weeks after initiation of ART. *APOA5* -1131T>C polymorphism was tested by real-time polymerase chain reaction. The mean age patients was 37.9 years; Most of patients were men (78.5%). The prevalence of total cholesterol (TC) ≥200 mg/dl, low-density lipoprotein cholesterol (LDL-c) ≥130 mg/dl, high-density lipoprotein cholesterol (HDL-c) <40 mg/dl, triglyceride (TG) levels ≥150 mg/dl and overall dyslipidemia at week 48

was 37.2%, 45.5%, 32.2%, 41.3% and 71.9%, respectively. At week 48, the mean of TC, LDL-c, TG and fasting glucose showed significant increase while HDL-c level showed significant decrease, compared with baseline. For *APOA5* -1131 T>C genotyping study, 58.7% were homozygous TT, 33.9% were heterozygous TC and 7.4% were homozygous CC. The median of triglyceride level of TT, TC and CC were 120 mg/dl, 157 mg/dl and 171 mg/dl, respectively (P=0.036). In conclusion, treatment with efavirenz-based ART was associated with dyslipidemia among HIV and tuberculosis co-infected patients. *APOA5* polymorphism may help the physician to select an appropriate treatment among these patients.

INTRODUCTION

Human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) are still an enormous public health problem. Joint United Nations Program on HIV/AIDS (UNAIDS) estimates approximately 36.7 million persons were living with HIV, 2.1 million were newly infected, and 1.1 million died from their infection in 2015 (1). Fortunately, the introduction of antiretroviral therapy (ART) from 1996 has dramatically reduced the morbidity and mortality of HIV-infected individuals (2). Highly active antiretroviral therapy (HAART) is the core of treatment for HIV-infected individuals which composed of three drugs. World Health Organization (WHO) has recommended the effective treatment of HIV which composed of 2 nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a boosted protease inhibitor (PI) (3).

Efavirenz is a NNRTI which has recently been recommended as first-line regimen in many guidelines (3, 4). From a previous study, efavirenz showed high efficacy and safety compared with other NNRTIs (5). Efavirenz, however, also has variable adverse effects (6). Dyslipidemia is one of the adverse effects that was reported in HIV-infected patients. Even dyslipidemia

has often been found in patients who received PIs but there are some reports of dyslipidemia in a NNRTIs group. Previous studies showed triglyceride (TG) tended to increase with efavirenz while nevirapine tended to decrease (7). In a double non-nucleoside study (2NN), the efavirenz treatment group showed a significant increase of total cholesterol (TC), non-high-density lipoprotein cholesterol (HDL-c) and TG compared to the nevirapine treatment group. Conversely, the increase of HDL-c was significantly larger for patients receiving nevirapine (42.5%) than for patients receiving efavirenz (33.7%) (8).

Regardless of similar comparable demographic, immunologic, virologic characteristics and exposure of ART, dyslipidemia does not occur in all patients. Host genetic factors may elucidate this phenomenon. Previous studies revealed genetic polymorphisms of hypertriglyceridemia in HIV-infected patients. In Chinese HIV patients, *apolipoprotein A5 (APOA5)* -1131T>C and c.553G>T polymorphisms were significantly associated high pre-HAART TG levels (9). Carriers of *APOA5* -1131T>C and c.553G>T variants may have an increased risk of PI-induced hypertriglyceridemia in Taiwanese HIV-infected patients (10). In addition, the study from Spain found that carriers of the *APOA5* -1131 C allele had consistently higher TG level than non-carriers at different time points of study period in HIV-infected patients receiving PIs (11). These evidences may suggest the *APOA5* gene polymorphisms was associated with the risk of abnormal lipid levels in HIV-infected patients.

APOA5, a gene which is responsible for triglyceride metabolism in the synthesis, metabolism, and clearance of triglyceride-rich lipoprotein (12). *APOA5* -1131T>C polymorphism, the single nucleotide polymorphism (SNP) in the promoter region, has been recognized as a significant contributor to hypertriglyceridemia in a various healthy populations (13). To our knowledge, HIV-infected patients who treated with HAART may be predisposed to a lipid

profile, associated with cardiovascular risk. HAART-associated dyslipidemia was influenced by the various forms of genetic polymorphisms which was similar to the non-HIV adults (14). However, there is a little information about the relationship between genetic polymorphisms *APOA5* -1131T>C and dyslipidemia in Thai HIV/TB co-infected patients who received efavirenz-based ART. The aim of this study was to investigate the association between *APOA5* -1131T>C polymorphism and dyslipidemia in Thai HIV/TB co-infected patients who received efavirenz-based ART.

MATERIALS AND METHODS

Subjects

This study was a retrospective cross-sectional cohort study. It was nested within the previous study (15). One-hundred and twenty-one Thai HIV/TB co-infection patients were recruited at the Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi from October 2009 to May 2011. The inclusion criteria were HIV-infected adult patients (18-65 years of age) and had diagnosed with active tuberculosis by clinical features, positive acid-fast staining and/or positive culture for *Mycobacterium tuberculosis*. Patients who did not provide lipid profile data for each week of observation were excluded.

All subjects were on an ART which was composed of 2 NRTIs and a NNRTI (tenofovir 300 mg, lamivudine 300 mg and efavirenz 600 mg) at bedtime. All subjects also received rifampin-based anti-tuberculosis treatment. The standard anti-tuberculosis regimen (isoniazid, rifampin, pyrazinamide, and ethambutol) was initiated for the first 2 months and was followed by 2 drugs, isoniazid and rifampin, for the subsequent 4 to 7 months based on clinical outcome.

Clinical characteristics and laboratory parameters collection

Clinical characteristics and laboratory parameters of patient (gender, age, body weight, blood pressure, CD4+ cell count, plasma HIV-RNA levels, lipid profile, fasting glucose) were collected at baseline (before starting ART) and 48 weeks (after initiation of ART). The patients were followed until 48 weeks after initiation of ART to examine lipid parameters. Lipid profiles were measured by an automated chemistry analyzer (Cobas Integra 400, Roche Diagnostics, USA). CD4+ cell counts were determined by flow cytometry. Plasma HIV-1 RNA levels were assessed by COBAS AmpliPrep/COBAS TaqMan HIV-1 test real-time polymerase chain reaction (PCR), (Roche Molecular Systems Inc, Branchburg, NJ).

***APOA5* -1131T>C (rs662799) SNP genotyping study**

All subjects were collected the EDTA blood sample and submitted to the Laboratory for Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital, Mahidol University for SNP genotyping study. Genomic DNA from blood samples were extracted by a MagNA Pure Compact instrument (Hoffman-La Roche Ltd., Basel, Switzerland). Genotyping method for *APOA5* -1131T>C (rs662799) was performed using TaqMan® real-time PCR (Applied Biosystems, CA, USA).

Dyslipidemia was defined by using the National Cholesterol Education Program Adult Treatment Panel III guideline (16). The cutoff points are TC $\geq$200 mg/dl, LDL-c $\geq$130 mg/dl, HDL-c $<$40 mg/dl, TG $\geq$150 mg/dl and hyperglycemia as glucose $\geq$110 mg/dl. Patients who had one or more abnormal lipid parameters were considered as dyslipidemic.

Statistical analysis

Demographic and laboratory data were expressed as mean and standard deviation for parametric data or median and interquartile ranges for non-parametric data. Paired t-test was used for comparing the lipid and glucose levels at baseline and 48 weeks of observation. The prevalence of dyslipidemia at week 48 in HIV/TB co-infected patients receiving efavirenz-based ART was expressed as percent. The association between lipid levels and *APOA5* -1131T<C (rs662799) polymorphisms at week 48 was determined using the Kruskal-Wallis test. Data were analyzed by using SPSS version 18 for Windows (SPSS, Inc., Chicago, IL, USA). *P-value* of < 0.05 was considered to be statistically significant.

This study was approved by the human research ethics committee of the Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand (MURA2016/517).

RESULTS

Baseline clinical characteristics and laboratory parameters of patients

At 48 weeks of observational period, 121 HIV/TB co-infected patients were enrolled into the study. All patients were treated with tenofovir (300 mg), lamivudine (300 mg), and efavirenz (600 mg). Baseline clinical characteristics and laboratory parameters were shown in Table 1. Most of patients were male (78.5%). The mean of age, bodyweight, blood pressure (systolic/diastolic) were 37.9 years, 54.9 kg and 117.3/74.5 mmHg. The median of CD4+ cell count and plasma HIV-1 RNA levels were 44 /mm^3 and 5.8 log copies/ml. The lipid profile and fasting glucose levels were desirable at baseline. The frequencies of the wild type, heterozygous and homozygous mutant of the *APOA5* -1131T>C polymorphism were 58.7%, 33.9% and 7.4%, respectively.

Table 1 Baseline clinical characteristics and laboratory parameters of HIV/TB co-infected patients receiving efavirenz-based ART (N = 121)

Characteristics	Values
Demographic data	
Gender	
Male N (%)	95 (78.5)
Female N (%)	26 (21.5)
Age (years), Mean ± SD	37.9 ± 8.7
Bodyweight (Kgs.), Mean ± SD	54.9 ± 9.7
Blood pressure	
Systolic (mmHg), Mean ± SD	117.3 ± 13.8
Diastolic (mmHg), Mean ± SD	74.5 ± 11.8
CD4+ cell count (cell/mm^3), Median (IQR)	44.0 (17.0-114.5)
Plasma HIV-RNA (log copies/ml), Median (IQR)	5.8 (5.4-6.3)
Genotyping study	
APOA5 -1131T>C (rs662799), N (%)	
TT	71 (58.7)
TC	41 (33.9)
CC	9 (7.4)

Kgs, kilograms; mmHg, millimeter of mercury; SD, standard deviation; IQR, interquartile range; *APOA5*, apolipoprotein A5

Dyslipidemia and glucose levels changes

This study showed a high prevalence of dyslipidemia in HIV/TB co-infected patients who received efavirenz-based ART. The prevalence of TC ≥200 mg/dl, LDL-c ≥130 mg/dl, HDL-c <40 mg/dl, TG levels ≥150 mg/dl and overall dyslipidemia at week 48 was 37.2%, 45.5%, 32.2%, 41.3% and 71.9%, respectively, as shown in Figure 1. The lipid and glucose levels

change in normal baseline HIV/TB co-infected patients were observed as shown in Table 2. The mean of TC, LDL-c, TG and fasting glucose showed significant increase compared with baseline (159.6 vs 179.7 mg/dl; $P<0.001$, 93.3 vs 116.8 mg/dl; $P<0.001$, 115.1 vs 134.7 mg/dl; $P=0.015$, and 90.1 vs 98.4 mg/dl; $P<0.001$, respectively). While the HDL-c level showed significant decrease compared with baseline (58.7 vs 51.2 mg/dl; $P=0.020$).

Table 2 Lipid and glucose level change at week 48 in normal baseline HIV/TB co-infected patients receiving efavirenz-based ART

Parameters (Mean±SD)	Baseline	48 Weeks	*P*-value*
Total cholesterol (mg/dl), N = 85	159.6±29.2	179.7±33.6	<0.001
LDL-Cholesterol (mg/dl), N = 88	93.3±29.5	116.8±33.5	<0.001
HDL-Cholesterol (mg/dl), N = 80	58.7±21.0	51.2±16.9	0.020
Triglyceride (mg/dl), N = 60	115.1±20.7	134.7±58.5	0.015
Fasting glucose (mg/dl), N = 106	90.1±9.6	98.4±13.7	<0.001

SD, standard deviation; LDL, low density lipoprotein; HDL, high density lipoprotein

**P-value* was calculated by paired-sample t-test. Significant difference was determined as *P-value* < 0.05

APOA5 SNP genotyping study

Figure 2 showed the association between TG level and *APOA5* -1131T>C (rs662799) polymorphism at week 48. The median of triglyceride level of the wild type, heterozygous and homozygous mutant of the *APOA5* -

1131T>C (rs662799) polymorphism were 120 mg/dl, 157 mg/dl and 171 mg/dl, respectively (*P*=0.036). From this study we found that patients who carried heterozygous TC and homozygous CC were borderline high TG level (41.3%). However, the association of *APOA5* -1131T>C polymorphism and other lipid parameters was not found as shown in Table 3.

Table 3 The relationship of *APOA5* -1131T<C (rs662799) polymorphisms and lipid levels at week 48 (N=121)

Parameters, Median (IQR)	*APOA5* -1131T<C (rs662799) polymorphisms			*P-value**
	TT	TC	CC	
Total cholesterol (mg/dl)	194.0 (162.0–219.0)	184.0 (156.0 – 225.5)	180.0 (162.5–251.0)	0.673
LDL-cholesterol (mg/dl)	130.0 (100.0–152.0)	114.0 (90.0 – 149.5)	103.0 (98.0 – 186.5)	0.585
HDL-Cholesterol (mg/dl)	45.0 (40.0 – 56.0)	43.0 (36.0 – 57.0)	51.0 (42.5 – 55.0)	0.518
Triglyceride (mg/dl)	120.0 (95.0 - 162.0)	157.0 (109.5 – 187.5)	171.0 (125.0 –202.0)	0.036

IQR, interquartile range; *APOA5*, apolipoprotein A5; LDL, low-density lipoprotein; HDL, high-density lipoprotein

* *P-value* was calculated by Kruskal-Wallis test. Significant difference was determined as *P-value* < 0.05

DISCUSSION

Our study was designed to investigate the association between *APOA5* -1131 T>C polymorphism and dyslipidemia in HIV and tuberculosis co-infected patients receiving efavirenz-based ART. This study suggested that there is a potential influence of *APOA5* -1131T>C polymorphism on TG levels while the other lipid parameters (TC, LDL-c and HDL-c) did not

show the association. To date, many studies have reported the association between *APOA5* -1131T>C polymorphism and hypertriglyceridemia in both healthy populations and HIV-infected patients receiving PI-based therapy. Although this was studied in HIV/TB co-infected patients receiving NNRTI-based ART, the results were consistent with previous studies (9-11, 13). The comparison of TG levels according to the genotype, carrier of *APOA5*-1131 C allele was significantly associated with higher TG level compared with T allele (11) . In this study, we also found that carriers of C allele (both heterozygous and homozygous mutant) had a TG level above the desirable condition. However, the study in type 2 diabetes mellitus patients in China did not find the association between *APOA5* - 1131T >C polymorphism and hypertriglyceridemia (17). This may suggest other factors (different populations, environmental influences, individual lifestyle) could play a role in this event.

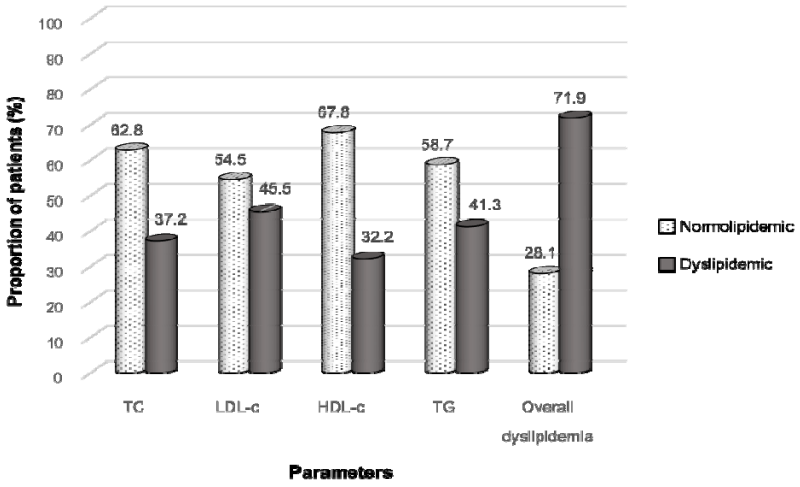

Figure 1 Prevalence of dyslipidemia at week 48 in HIV/TB co-infected patients receiving efavirenz-based ART (N=121)

The prevalence of overall dyslipidemia in this study was high at week 48 of observation (71.9%). This was similar to the cross-sectional study of dyslipidemia in Cameroonian HIV-infected patients receiving first-line ART (18). At 48 weeks of efavirenz-based ART initiation, the mean of TC, LDL-c, TG and fasting glucose showed significant increase compared with baseline; while the mean of HDL-c showed significant decrease. There are some publications showed a different pattern of abnormal lipid levels. The study from India (19) found the means of TC, LDL-c and HDL-c levels increased at each time point of 6 months and 12 months while the means of TG levels were not different from baseline. Another study of dyslipidemia in HIV-infected patients receiving NNRTI-based ART found elevated TG (26%) and low HDL-c (55%) prior to ART initiation. At 6 months, TG decreased and HDL-c increased while TC and LDL-c increased at each time point (20). This may indicate the effect of ART on lipid profile in these patients. In addition, previous study showed that higher plasma efavirenz concentrations are associated with higher plasma lipid and glucose concentrations (21). However, the mechanism remains unclear. Although the lipid levels were not elevated above the desirable condition, the pattern of elevated levels of lipids in this study tended to increase the risk of dyslipidemia which is an important risk factor for the development of cardiovascular disease (22).

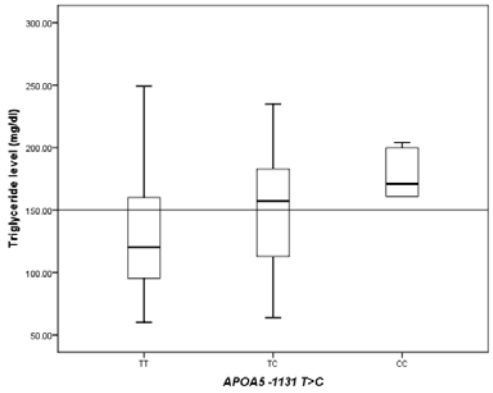

Figure 2 The association between triglyceride level and *APOA5* -1131T<C (rs662799) polymorphisms at week 48 (P = 0.036)

This study has several limitations. This was a retrospective cross-sectional study; the data including dietary history data and individual lifestyle could not be collected. Some clinical and laboratory data were lost during the study resulting in the sample size that was rather small.

In conclusion, treatment with efavirenz-based ART was associated with dyslipidemia among HIV and tuberculosis co-infected patients. *APOA5* -1131 T>C polymorphism could play a predictive role among these patients. Genetic information could assist clinician to select the appropriate treatment in this population.

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KEYWORDS: HIV, Efavirenz, Apolipoprotein A5, Dyslipidemia

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