APOLIPOPROTEIN E GENE POLYMORPHISMS AND PLASMA LIPIDS IN PERSONS LIVING WITH HIV: A CROSS SECTIONAL STUDY

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ABSTRACT

Background and Objective: **A major modifiable risk factor for atherosclerotic cardiovascular disease is abnormalities in lipid and lipoprotein metabolism which are frequently seen in HIV as well as its treatment. Apo-E is a protein that is important in plasma lipid homeostasis and its genetic alleles have been shown to contribute to lipid abnormalities. We examined for the effect of Apo-E gene polymorphisms on plasma lipid levels in PLHIV on protease inhibitor therapy.**

Methods: **This was a cross-sectional study conducted among adult persons living with HIV. Lipid profile, Apo-B and Apo-A were measured in fasting plasma. Amplification and analysis of Apo-E genotypes were determined using the Seeplex Apo-E ACE genotyping kit. Differences in quantitative values were compared with non-parametric analysis methods.**

Results: **Eighty-four persons were recruited into the study, 75% of whom were virally suppressed. The 3 homozygous genotypes had significantly different levels of low-density lipoprotein cholesterol (LDL-C), Apolipoprotein B (Apo-B) and Apolipoprotein A1 (Apo-A1). Persons with apo ε2/ε2 had higher LDL-C compared to those with apo ε3/ε3 (3.26 (3.61) mmol/L vs. 2.76 (1.28) mmol/L, p = 0.010). Those with apo ε4/ε4 had lower Apo-A1 compared to those with apo ε3/ε3 (0.84 (0.48) g/dL vs. 1.27 (0.70) g/dL, p =0.009). Compared with the same group, the heterozygous genotype, apo ε2/ε3 had lower triglyceride levels :1.33 (0.65) mmol/L vs. 1.86 (1.11) mmol/L, p = 0.045.**

Conclusion: **Polymorphisms in the Apo-E gene may have significant influences on plasma lipid and apolipoprotein levels in PLHIV on PI therapy. This may have implications for the assessment of risk for cardiovascular disease.**

Keywords: Polymorphisms, Genotypes, Dyslipidaemia, Protease Inhibitor, HIV

INTRODUCTION

Persons living with HIV (PLHIV) have an increased risk of atherosclerotic cardiovascular disease (ASCVD). The relative risk of Cardiovascular Disease (CVD) in this population is at least 50% higher than observed in the general population.[1,2] This is a consequence of an increased prevalence of traditional risk factors for CVD in PLHIV as well HIV specific factors which include the chronic inflammation and immune activation caused by the disease as well as side effects of some antiretroviral drugs. An effective risk management response requires primary prevention efforts at the level of the individual. At the heart of efforts is the identification of individuals at high risk who may then be offered appropriate intervention.[3]

Interventional options aimed at primary prevention focus on the modification of the traditional risk factors. A major modifiable risk factor for CVD is abnormalities in lipid and lipoprotein metabolism. HIV as well as its treatment are associated with dyslipidemia. HIV is characterized by low levels of Total Cholesterol (TC), High-Density Lipoprotein–Cholesterol (HDL-C) and Low-Density Lipoprotein-Cholesterol (LDL-C) and in more advanced disease with increased Triglycerides (TG). All classes of antiretroviral therapy (ART) induce dyslipidemia but regimens which include protease inhibitors (PI), second line ART agents in Nigeria, have been reported to have greater than a 3-fold risk of dyslipidemia compared to patients on

regimens that did not include this class of drug.[4] Hypercholesterolemia and hypertriglyceridemia have been noted to be present in over 50% of PLHIV after 2 years of PI based therapy.[5] Other factors may, however, be involved in the metabolic and lipid alterations observed in PLHIV on ART because not all of the patients exposed to the same regimen are similarly affected[6]. Such factors may include virologic, individual immunologic features as well as genetic factors.

Apo-E is a polymorphic protein that is central to the regulation of plasma lipid levels. The major way it serves this role is by mediating the binding of remnant lipoproteins in the plasma to specific cell-surface receptors which internalize Apo-E-containing lipoprotein particles.[7] It has three major isoforms Apo-E2, Apo-E3, and Apo-E4 which are the gene products of three alleles, ε2, ε3 and ε4, of a single gene locus located on chromosome 19.[8] The 3 isoforms of Apo-E have differential effect on lipid metabolism due to their affinity for the receptors in the clearing of the remnant lipoproteins with Apo-E2 having reduced and Apo-E4 having increased binding ability to the receptor compared to Apo-E3, the wild type.[7] The presence of Apo-E alleles have been shown to contribute significantly to the variability of lipid and apolipoprotein concentrations.[9]

The manifestation of the dyslipidemia associated genetic alterations in the Apo-E gene has however been shown to frequently require an additional factor to precipitate the occurrence of dyslipidemia. The additional effect of diseases like diabetes, hypothyroidism and chronic kidney disease have been associated with the development of dyslipidemia in persons homozygous for a variant Apo-E gene.[10,11] Given the effects of HIV and PI therapy on lipid metabolism we aimed to examine for the effect of polymorphisms of the Apo-E gene on the plasma lipid and apolipoprotein levels among a group PLHIV on protease inhibitor therapy.

MATERIALS AND METHODS

Study design/participants

This was a cross-sectional study conducted in the adult retroviral clinic of the Infectious Diseases Institute of the University of Ibadan, Ibadan between March and June 2019. Consecutively consenting HIV infected adults on protease inhibitor (PI) therapy were recruited into the study. Persons with a baseline history of CVD or on statins were excluded. A structured questionnaire was used to obtain demographic indices, medical history, HAART history, as well as current CD4 and HIV Viral load. All persons who presented within the study period were recruited for the study.

Sample collection processing and analysis

Ten (10) mL of blood was drawn from each patient after a 12 hour fast. Five (5) mls of blood was dispensed into a plain bottle for lipid profile and the remaining 5 ml in EDTA bottle for Apo-E studies. Clotted blood in the plain bottles was allowed to retract and the samples were then centrifuged at 3000 g for 15 minutes and sera obtained decanted into new plain bottles. Fasting blood samples were analyzed for serum total cholesterol (TC), HDL-C, TG, and LDL-C levels with ready to use reagents from Fortress Diagnostics Limited, Antrim, United Kingdom (Product Code: BXC0261, BXC0422A, BXC0271, and BXC0431 respectively). Plasma levels of Apo-B and Apo-A1 were measured by immunoturbidimetry with ready to use reagents supplied by Fortress Diagnostics Limited, Antrim, United Kingdom (Product Code: BXC0412 and BXC0411, respectively). Amplification and analysis of Apo-E genotypes were determined using the Seeplex apoE ACE genotyping kit. The Seeplex Apo-E ACE genotyping kit is a polymerase chain reaction DNA amplification technique that uses a proprietary oligo technology called DPO (Dual Priming Oligonucleotide). It is designed to identify the six common apoE genotype in one PCR step. Identification of Apo-E genotype was done using electrophoresis. 5μL of PCR products and size markers was separated with electrophoresis using 2% agarose gel. Fragment sizes were estimated by comparison with known size markers. Apo-B/Apo-A1 and LDL-C/Apo-B ratio and were calculated by diving the plasma concentrations (both in mg/dL) of the parameters.

Statistical analysis

Values are presented as median (interquartile range). Comparison of values were done with either Mann Whitney U or Kruskal Wallis Test as appropriate. Linear regression was employed to evaluate the relationship between Apo-E genotypes and clinical parameters. Significance was set at $p < 0.05$.

Ethical Consideration

The study was carried out in accordance with the declaration of Helsinki and ethical clearance was obtained from the University College Hospital / University of Ibadan Joint Ethics Review Committee (UI/UCH EC: UI/EC/18/0328).

RESULTS

Eighty-four (84) consecutively consenting HIV-infected persons on protease inhibitor (PI) therapy were recruited into the study. Their demographic and clinical characteristics are shown in Table 1. Majority of the participants were female, and their ages ranged from 32 to 70 years with 66.6% aged below 50 years. Duration of HIV diagnosis, highly active antiretroviral

Table 1: Characteristics of study population

Variable	Total	Apo ε3/ε3	Apo ε2/*	Apo ε4/*	p-value
Number, (%)	84 (100)	36 (42.9)	18 (23.8)	30 (33.3)	
Age, yrs	47.0 (10.0)	48.0 (14)	45.0 (8)	44.0 (14)	0.111
Female, n (%)	66 (78.6)	28 (77.8)	16 (88.9)	22 (73.3)	0.46
HIV Diagnosis, yrs	11.5 (5.0)	12.0 (4)	12.0 (6)	10.0 (6)	**0.007**
HAART use, years	10.5 (6.0)	11.4 (7.0)	11.0 (7.0)	8.0 (6.0)	**0.007**
PI use, years	8.0 (4.0)	8.8 (4.5)	9.0 (3.0)	7.0 (6.0)	**0.016**
BMI (kg/m²)	24.7 (6.7)	24.7 (8.3)	23.8 (7.0)	24.7 (3.9)	0.181
VL (cps/mL)	15 (303)	20.5 (303)	33.0 (115)	10.0 (511)	0.804
CD4 (cells/uL)	397 (543)	391.5 (537)	515.5 (580)	318.0 (515	0.051
TC (mmol/L)	4.6 (2.1)	4.5 (1.3)	4.8 (2.2)	5.3 (3.8)	0.522
TG (mmol/L)	1.7 (1.1)	1.9 (1.1)	1.4 (1.0)	1.6 (1.6)	0.129
HDL-C (mmol/L	1.1 (1.1)	1.0 (1.2)	1.1 (1.4)	1.2 (0.7)	0.475
LDL-C (mmol/L)	2.7 (1.9)	2.7 (1.3)	3.1 (1.4)	2.7 (3.6)	0.490
Apo-B (g/dL)	1.1 (0.5)	1.2 (0.2)	1.2 (0.4)	1.2 (1.1)	0.481
Apo-A1 (g/dL)	1.2 (0.6)	1.3 (0.7)	1.3 (0.8)	1.2 (0.5)	0.869
Apo-B/Apo-A1 ratio	0.9 (0.8)	0.9 (1.6)	0.9 (0.3)	1.0 (1.2)	0.478
LDL-C/Apo-B (mmol/g)	2.5 (2.4)	2.1 (2.4)	2.5 (0.9)	3.1 (4.2)	0.304

Values are median (interquartile range). HAART - Highly active antiretroviral therapy; PI – protease inhibitor; BMI – body mass index; VL – Viral Load; CD4 – CD4 positive T lymphocytes; TC - Total Cholesterol; TG – Triglycerides; HDL-C – High Density Lipoprotein Cholesterol; LDL-C - Low Density Lipoprotein Cholesterol; LDL-C - Low Density Lipoprotein Cholesterol; Apo-B – Apolipoprotein B; Apo-A1 - Apolipoprotein A1. Apo £2/- Apo £2 and any other isoform; Apo £3/ *- Apo £3 and any other isoform.*

therapy, and protease inhibitor therapy ranged from 2 – 17 years, 2 – 15 years, and 1.25 – 12 years, respectively. Over 80% of the participants had been on PI for more than 5 years. Viral suppression (less than 1000c copies/ mL) had been achieved in over 75% of the participants while 48 (57.1%) persons had CD4 values less than 500 cells/ìL. Body Mass index (BMI) in the underweight, normal weight, and overweight categories occurred in 4(4.8%), 46 (54.8%), and 34 (40.5%), respectively.

and Apo-B were significantly higher in those with apo ε2/ε2 compared to those with apo ε3/ε3, 5.39 (4.88) mmol/L v 4.54 (1.27) mmol/L, p = 0.02; 3.26 (3.61) mmol/L vs. 2.76 (1.28) mmol/L, p = 0.010, and 1.23 (1.21) mg/dL vs. 1.01 (0.21) 010 mg/dL, p=0.008, all respectively. Persons with apo ε4/ε4 had a lower Apo-A1 compared to those with apo: ε3/ε3 0.84 (0.48) g/dL vs. 1.27 (0.70) g/dL, p =0.009. A comparison of the values observed in persons who are apoε3 homozygous against those are heterozygous

Table 2: Lipid and Apolipoprotein parameters in persons homozygous for apoε gene

	ε2/ε2	ε3/ ε3	ε4/ε4	
Number	6	36	8	
TC (mmol/L)	4.0 (1.4)	4.5 (1.3)	4.5 (2.9)	0.059
TG (mmol/L)	1.3 (0.7)	1.9 (1.1)	1.4 (1.1)	0.129
HDL-C (mmol/L)	1.1 (1.5)	1.0 (1.2)	0.7 (0.9)	0.103
LDL-C (mmol/L)	2.6 (1.6)	2.7 (1.3)	3.0 (2.9)	0.048
Apo-B (g/dL)	1.0 (0.4)	1.0 (0.2)	1.1 (0.7)	0.039
Apo-A1 (g/dL)	1.2 (1.0)	1.3 (0.7)	0.8 (0.5)	0.018
Apo-B/Apo-A1 ratio	0.9 (0.1)	0.9 (0.9)	1.2 (1.2)	0.263
LDL/Apo-B (mmol/g)	2.3 (0.9)	2.1 (2.4)	3.2 (4.3)	0.370

LDL-C – Low Density Lipoprotein Cholesterol; Apo-B – Apolipoprotein B; Apo-A1 – Apolipoprotein A1.

Table 2 compares the levels of the measured and calculated lipid and apolipoprotein parameters as observed for persons who are homozygous for the apo ε alleles. The levels of LDL-C, Apo-B and Apo-A1 were significantly different across the 3 groups. Subgroup analyses showed that levels of TC, LDL-C

for apoε3 (apo ε2/ε3 and ε3/ε4) was done. Significant observations were lower TG levels in persons with apo ε2/ε3 {1.33 (0.65) mmol/L vs. 1.86 (1.11) mmol/L, p = 0.045} and higher TC levels in persons with ε3/ε4 {6.04 (4.19) mmol/L vs. 4.54 (1.27) mmol/L, p = 0.01}.

DISCUSSION

The presence of mutant alleles for the Apo-E gene has significant influence on lipid and apolipoprotein levels. The specific lipid and apolipoprotein affected and the nature of the affectation is dependent on the mutant allele present and whether the allele is present in a homozygous or heterozygous state. Homozygosity for apoε2 is the primary genetic defect for the development of type III hyperlipoproteinemia which is characterized by elevated levels of total cholesterol and triglycerides (TGs) due to high plasma levels of chylomicron and very low-density lipoprotein (VLDL) remnants enriched in cholesterol esters.[12] Only 1% of individuals with apoε2/ε2 profile, however, develop the typical severe hypercholesterolemia and hypertriglyceridemia that is typical of the disease. Protease inhibitor therapy for HIV has been proposed as a possible modulating secondary factor.[10,13] A possible mechanism of the unmasking of type III hyperlipoproteinemia by protease inhibitors relates to the sharing of a 63% amino acid sequence homology with the LDL-receptor-related protein.[14] This results in interference with the hepatic uptake of remnant lipoproteins with consequent severe hypercholesterolemia and hypertriglyceridemia. Amongst our population of HIV persons on PI therapy who are homozygous for apoε2, the levels of cholesterol and triglycerides while mostly elevated was not typical of type III hyperlipoproteinemia. This seemingly surprising results has some support in the findings by Shahmanesh *et al.*[15] among a group of PLHIV on PI-based therapy. The presence of type III hyperlipoproteinemia among their patients was not related to the apoε2/ε2 genotype. This suggests that in the presence of the mutant homozygous state, the presence of a known secondary factor, in this case, PI therapy, may not uniformly lead to disease expression. The role of secondary genetic alterations (gene to gene interactions) affecting Apo-B metabolism and LDL-receptors as possible mechanisms to explain this inconsistent association apoε2/ε2 with type III hyperlipoproteinemia has been demonstrated in animal and human studies.[16–18]

The apoε4/ε4 genotype, beyond its classical association with the development of late-onset sporadic Alzheimer's disease[20] is associated, along with apoε3/ε4, with a higher incidence of CHD.[20] Its proatherogenic effect includes a small but significant decrease in HDL levels.[21] Among this study participants, the plasma concentration of HDL-C was not significantly lower than for apoε3/ε3, but we did demonstrate significantly lower levels of Apo-A1, the structural apolipoprotein of HDL. HDL-C and Apo-AI are both surrogate measures of the lipoprotein HDL,[22] and have shown similar ability in predicting cardiovascular disease.[23] There are methodological advantages to the use of Apo-A1 over HDL-C. Principal among these reasons is that assays for Apo-A1 have been internationally standardized with WHO-IFCC, reference materials available and assays achieving methodological coefficient of variation of <5%. These methodological advantages may explain why Apo-A1 may be more sensitive to detect the small changes reported for persons with apoε4 in a small size study population as ours. Beyond these analytical reasons, reductions in HDL-C could be due to a down-regulation of hepatic Apo-A1 transcription and/or greater clearance of HDL-C from the circulation.[24] Our findings, of a significantly low Apo-A1 and not HDL-C, suggest a mechanistic explanation that the effect of apoε4/ε4 might be the downregulation of transcription. This is also suggested by the role the variant plays in the pathogenesis of other diseases where it has been shown to function as a transcription factor.[25]

The concentration of Apo-B among the participants in this study was significantly influenced by polymorphisms in the Apo-E gene. Individuals with ε2 and ε4 isoforms had lower and higher levels, respectively, of Apo-B compared to those without those isoforms. This mirrors the report of Evans *et al.* amongst a group of healthy Chinese males.[26] They observed that Individuals carrying the ε2 allele had lower and the ε4 allele had higher levels of Apo-B than individuals homozygous for the ε3 isoform. These findings may be explained by the findings of Welty *et al.* in their study of the effects of Apo-E polymorphisms on the kinetics of Apo-B. They found that individuals with a single allele of apoε4 demonstrated lower fractional catabolism of apoB-100 along with increased conversion of VLDL to LDL.[27] Several mechanisms have been proposed to explain the low Apo-B levels in ε2 homozygotes. These include an upregulation of the hepatic LDL receptor following decreased delivery of cholesterol to the liver as a result of defective binding of Apo-E2 containing lipoproteins and a reduced competitiveness between the Apo-E2 containing lipoproteins and containing LDL for the LDL receptor causing an increased removal of the Apo-B containing lipoproteins.[28-30]

Limitation of the study

A limitation of this study is the relatively few numbers of persons who are homozygous for the ε2 and ε4 alleles. This limits the generalizability of the conclusions derivable from our data. This reflects the frequency of the occurrence of the alleles in the general population as well the sample size of the study.

CONCLUSION

We have demonstrated polymorphisms in the Apo-E gene have important influences on the pattern of plasma lipid and apolipoprotein levels. This may have implications for ARV therapy decisions as well assessment of risk for cardiovascular disease.

Competing interests

The authors declare no competing interest

Authors' contributions

MAK: conceived the study, oversaw laboratory analysis, performed data analysis, contributed to interpretation of results, and drafted the manuscript; OTB: contributed to overall study design, laboratory analysis, and interpretation of data and review of manuscript; NSN: contributed to overall study design, field work, laboratory analysis, interpretation of data, and critical review of the manuscript. OOO: contributed to the field work and gathering of data OAO: contributed to interpretation of results and drafted the manuscript KSA: conception of the study and its design and final approval of manuscript. OAA: conception of the study and its design and final approval of manuscript. All the authors have read and agreed to the final manuscript.

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