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Effect of EcoR1 Polymorphism of the Apoprotein B100 Gene (Apo B 4154G>A) on Serum Lipid Profile in ART-Naive PLHIV

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Abstract: Many people living with HIV (PLHIV) have dyslipidemia and influence of genetic predisposing factors are suspected. Some apolipoprotein genes polymorphisms are recognized as susceptibility factors, especially EcoR1 polymorphism of Apo B100 gene, main atherogenic lipid metabolizing apoprotein. The objective was to investigate the link between EcoR1 polymorphism of Apo B100 gene (Apo B 4154G>A) and the occurrence of dyslipidemia in ART-naive PLHIV. We did a cross-sectional study which included 32 HIV-negative controls and 23 PLHIV, above 18 years old, with at least one serum lipid and apoprotein disorder. Polymorphism testing was performed by PCR-RFLP and allele distribution study was performed using Hardy Weinberg equilibrium. As results, we found that the subjects were predominantly young males in both groups. BMI was higher in PLHIV. There were lipid disorders common to both PLHIV and HIV- subjects. However, PLHIV were distinguished by hypoapoproteinemia Apo A1 and HDL hypocholesterolemia. The 3 possible genotypes of EcoR1 polymorphism were found in both groups with a predominance of the mutant genotype in PLHIV (85.7% vs 14.3%). Also, the mutant allele frequency was higher in PLHIV (27.1% vs 6.2%). Regardless of HIV status, the mutant allele was more frequent in people under 40 years old, women and people with high BMI. In PLHIV, Hardy Weinberg equilibrium was deviated in all subgroups with the mutant allele frequency higher than 10%. In the overall population, the mutant allele was more frequent in subjects with increased TG, LDL-C, Apo B100, Lp (a), and CT/HDL atherogenicity index and those with normal total cholesterol, decreased HDL-C and Apo A1. Taking into account HIV status, the mutant allele found was more frequent in PLHIV (14% to 32% versus 2% to 21%). In these PLHIV, the mutant allele was more frequent especially in cases of total hypercholesterolemia (28.1%), normal LDL cholesterolemia (26.7%), HDL hypocholesterolemia (27.3%), hypoapoproteinemia A1 (32.1%), hyperapoproteinemia B100 (28.1%), hyperapoproteinemia Lp (a) (28.1%), high atherogenicity indexes (23.7%). In conclusion, the distribution of EcoR1 polymorphism alleles at position 4154 in exon 29 of ApoB100 gene was not random in PLHIV people. The carrying of mutant allele was more frequent in PLHIV and associated to increased atherogenic apolipoproteins and decreased atherosclerotic protective apolipoproteins (Apo A1 and HDL-C). The link between EcoR1 and ART must be investigates.

Keywords: EcoR1 Polymorphism, Apolipoprotein B, Dyslipidemia, HIV, Restriction Enzymes

1. Introduction

Human immunodeficiency virus (HIV) infection remains a public health problem. In 2020, 37.6 million [30.2- 45.0 million] people were living with HIV worldwide [1]. Infected persons, treated or not by antiretroviral drugs, show metabolic disorders, dyslipidemia in particular, a risk factor for cardiovascular diseases [2, 3, 4]. This suggests that the infection itself has a deleterious metabolic effect [3, 5, 6]. Also, all PLHIV do not have dyslipidemia, raising the suspicion that host-related genetic predisposing factors have an influence on the occurrence of dyslipidemia. Several single nucleotide polymorphisms (SNPs) were associated with the occurrence of dyslipidemia, particularly in the apolipoprotein genes [7, 8, 9, 10]. It has been shown that HIV-associated dyslipidemia is accompanied by an increase in lipoproteins containing Apolipoprotein B (Apo B), particularly LDL, and high LDL cholesterol is known to be one of the predictive factors of cardiovascular risk [3, 5]. Thus, the Apo B100 gene is one of the most studied genes of interest; particularly the EcoR1 and Xba1 polymorphisms [7, 10, 11].

The objective of this study was to establish the link between EcoR1 polymorphism of the Apo B gene and changes in serum lipoprotein profiles in ART-naive PLHIV in Côte d'Ivoire.

2. Materials and Methods

2.1. Materials, Population and (Setting) Study Sites

The study population consisted of HIV-negative controls (HIV-) voluntary blood donors and PLHIV monitored at the National Blood Transfusion Center (CNTS) management center and showing a disturbed lipid report (classical lipids and apolipoproteinemia).

2.2. Methods

This was a cross-sectional study.

Sample collection and storage

Whole blood collected on EDTA tubes was sent to CeDReS where aliquots were stored at -80°C, and then lipoprotein parameters were measured. Sociodemographic, therapeutic and biological data (lipid report) were collected from the database of the first part of the study. Indeed, the first part of the study had already been carried out using these same samples [12, 13].

Ethical considerations

The recruited persons gave their informed consent to participate in the study. The study was conducted with the approval of the National Ethics and Research Committee of Côte d'Ivoire under the reference number: 3766/MSHP/30/July 2009.

Determination of the EcoR1 polymorphism of the ApoB100 gene

Primers used: The primer pair (SIGMA ALDRICH) used for PCR was: Bc2/Bc2R (sequence of interest on exon 29). The sequences were: Bc2: lot HA06611951 (5'-GAAGAG CCTGAAGACTGACT-3'), Bc2R: lot HA06611952 (5'-CTCGAAAGGAAGTGTAATCAC-3').

Extraction and study of extracts: The extraction of genomic DNA was performed on globular pellets with the Qiagen® kit technique. Extracts were stored at 4°C until amplification and then the rest frozen at -80°C for later use.

Amplifications: Amplifications were done by conventional PCR and the size of the sequence of interest was 480 bp. The PCR was performed with a 50 µL reaction mixture consisting of 1.5 µL of each of the primers at 10 µM, 5 µL of solution containing the 4 dNTPs at 2 µM, 1.5 µL of MgCl2 at 50 µM, 5 µL of 10X buffer, 0.5 µL of Taq polymerase at 5 IU/µL, 30 µL of molecular biology grade water and 5 µL of DNA PCR was performed on an ABI 2720 Thermal Cycler (Life Technologie). The PCR program consisted of an initial denaturation for 5 min at 95°C followed by 40 cycles of the following 3 steps: denaturation for 45 sec at 95°C, hybridization at 58°C for 45 sec and elongation at 72°C for 45 sec followed by a final elongation at 72 °C for 10 min and finally 4°C ad infinitum [12, 14]. Success of PCR was verified by electrophoresis on 1% agarose gel containing ethidium bromide (BET). In case of failure (no band on electrophoresis), the PCR was repeated. The amplicons were stored in the refrigerator (4-8°C) pending the RFLP step.

Enzymatic digestion and the search for polymorphism: After PCR, the amplification products were subjected to digestion with the restriction enzyme EcoR1 (G / AATTC) according to the predefined protocol [10]. After the digestion step, the DNA fragments were separated by electrophoresis on 1% agarose gel containing ethidium bromide (BET). The action of the enzyme generates 2 fragments of size 253 and 227 bp [11, 15] and indicates the presence of the G (wild type) allele of the 4154 G>A polymorphism of exon 29 of the ApoB100 gene (E+ allele). The absence of digestion of the sequence indicates the A allele (mutant) and results in a single 480 bp fragment G allele (wild).

Study of the distribution of alleles: It has been made by the Hardy-Weinberg equilibrium (HWE). Allelic frequencies are determined from the observed genotypic frequencies. Theoretical numbers of each genotype are then calculated and compared with the observed numbers from the chi-square (X^2) calculation. The X^2 is significant if $X^2 > 3.84$ [16, 17, 18]. Formulas used are:

Allelic frequencies:

$$p\ (E+) = (2x + z) / 2N \quad (1)$$

$$q\ (E-) = (2y + z) / 2N \quad (2)$$

Expected numbers:

$$E+/+ = Np^2;\ E-/- = Nq^2;\ E+/- = 2pqN \quad (3)$$

Calculated Chi-square:

$$X^2 = \sum [(\text{observed numbers} - \text{expected numbers})^2/\text{expected numbers}] \quad (4)$$

N: size of the study population, x: number of individuals with E+/+ genotype, y: number of individuals with E-/- genotype,

z: number of individuals with E+/- genotype

Statistical analysis of data

The collected data were analyzed using SPSS 16.0 software. The presence of DNA digestion was noted (+) and its absence (-). Quantitative variables were expressed on average ± standard deviation. Qualitative variables were expressed as percentages (%) and numbers (n). Comparisons were made using statistical tests: Student t-test for quantitative values and Chi 2 or Fisher exact test for qualitative values. The tests were considered significant at the risk $\alpha < 0.05$.

Hardy-Weinberg equilibrium (HWE) was tested by the 1-degree-of-freedom (1ddl) Chi-square test; the significance level was 3.84 ($X^2 > 3.84$).

3. Results

A total of 55 subjects were recruited: 23 ART-naive PLHIV (41.82%) and 32 HIV- control subjects (58.18%).

3.1. Presentation of the Population According to Sociodemographic Cardiovascular Risk Factors

There was a male predominance in both groups (sex ratio M/F: 4.3 in HIV- subjects and 1.3 in PLHIV) but there were no significant differences between the 2 groups regarding gender and age distribution. The mean BMI was higher in PLHIV than in HIV- subjects: 23.82 ± 3.37 versus 26.29 ± 5.4 (*p=0.046*). Also, high BMI values (> 25) were more frequent in PLHIV: n= 14 (63.6%) versus n= 11 (35.5%) (*p=0.043*).

3.2. Presentation of the Population According to Serum Lipid and Apoprotein Concentration Levels

Hypo-apoproteinemia A1 was more frequent in PLHIV (n=14 (60.90%)) compared to HIV- subjects (n=7 (21.9%)); *p= 0.002*. There was in both groups the predominance of hypertriglyceridemia (PLHIV: 19 (59.4%); HIV- subjects: n=16 (69.6%)), hyper-Apo B (PLHIV: 28 (87.5%); HIV- subjects: 16 (69.6%)), hyper-apoproteinemia Lp (a) (PLHIV: n=20 (62.5%); HIV- subjects: 16 (69.6%)) as well as high atherogenicity index: TC/HDL-C (PLHIV: n=17 (53.1%); HIV- subjects: n=19 (82.6%)) and ApoB/Apo A1: (PLHIV: n=19 (82.6%; HIV- subjects: n=25 (78.1%)). But the difference was significant only for the atherogenicity index TC/HDL-C *(p= 0.023)*.

3.3. Presentation of the Population According to the EcoR1 Polymorphism

The wild genotype (E+/+) predominated in both groups and all 3 genotypes were found in both (Table 1). The wild allele (E+) predominated in HIV- subjects (*p=0.002*) while the mutant allele (E-) predominated in HIV- subjects (Table 1). The HWE was deviated in both HIV- ($X^2 = 6.97$; *p = 0.008*) and PLHIV subjects ($X^2 = 19.20$*; p < 0.0001).*

Table 1. *Subject distribution according to geneotypes and alleles.*

	HIV- Subject N=32	**ART naive PLHIV N=23**	**p-value**
Genotypes			
Wild (E+/+)	29 (63.0%)	17 (37.0%)	
Mutant / Wild (E+/-)	2 (66.7%)	1 (33.3%)	NA
Mutant (E-/-)	1 (16.7%)	5 (83.3%)	
Alleles			
Wild allele (E+)	60 (93.8%)	35 (76.1%)	0.002
Mutant allele (E-)	4 (6,2%)	11 (23.9%)	

3.4. Distribution of Genotypes and Alleles According to Cardiovascular Risk Factors

Regardless of HIV status, genotype frequencies were not at HWE for any of the demographic factors. The mutant allele was more frequent in those under 40 years of age, women, and those with high BMI (Table 2). According to HIV status (Table 3), in the HIV- group, HWE was deviated in those under 40 years of age, males and those with high BMI. In PLHIV, the equilibrium was deviated in all subgroups with frequencies of the mutant allele in the proportions higher than 10%.

Table 2. *Genotypes and alleles distribution according to demographic cardio-vascular risk factors in the general population regardless of HIV infection status.*

Age Group (N=54)		
	≤ 40 years (n=29)	>40 years (n=25)
Genotypes		
Wild (E+/+)	24 (53.3%)	21 (46.7%)
Mutant / Wild (E+/-)	1 (33.3%)	2 (66.7%)
Mutant (E-/-)	4 (66.7%)	2 (33.3%)
Alleles		
Allele E+	49 (84.5%)	44 (88.0%)
Allele E-	9 (15.5%)	6 (12.0%)
HWE (X^2/p-value)	21.87 / <0.0001	9.65 / 0.0019
SEX (N=55)		
	Male (n=39)	Female (n=16)
Genotypes		
Wild (E+/+)	33 (71.7%)	13 (28.3%)
Mutant / Wild (E+/-)	2 (66.7%)	1 (33.3%)
Mutant (E-/-)	4 (66.7%)	2 (33.3%)
Alleles		
Allele E+	68 (87.2%)	29 (85.3%)
Allele E-	10 (12.8%)	5 (14.7%)
HWE (X^2/p-value)	23.16 / <0.0001	9.31 / 0.002
BMI (N=53)		
	≤ 25 kg/m² (n=28)	> 25 kg/m² (n=25)
Genotypes		
Wild (E+/+)	26 (57.8%)	19 (42.2%)
Mutant / Wild (E+/-)	1 (33.3%)	2 (66.7%)
Mutant (E-/-)	1 (20.0%)	4 (80,0%)
Alleles		
Allele E+	53 (94.6%)	40 (80.0%)
Allele E-	3 (5.4%)	10 (20.0%)
HWE (X2/p-value)	11.75 / 0.0006	14.06 / 0.0002

BMI: Body mass index, HWE: Hardy Weinberg Equilibrium.

Table 3. *Genotypes and alleles distribution according to demographic cardio-vascular risk factors and according to HIV infection status.*

	HIV- Subject		**ART naive PLHIV**	
Age Group (years) (N=54)				
	≤ 40 (n=18)	> 40 (n=13)	≤ 40 (n=11)	>40 (n=12)
Genotypes				
Wild (E+/+)	16 (57.1%)	12 (42.9%)	8 (47.1%)	9 (52.9%)
Mutant / Wild (E+/-)	1 (50.0%)	1 (50.0%)	0 (0.0%)	1 (100.0%)
Mutant (E-/-)	1 (100.0%)	0 (0.0%)	3 (60.0%)	2 (40.0%)
Alleles				
Allele E+	33 (91.7%)	25 (96.2%)	16 (72.7%)	19 (79.2%)
Allele E-	3 (8.3%)	1 (3.8%)	6 (27.3%)	5 (20.8%)
HWE (X^2/p-value)	7.290 / 0.0069	0.021 / 0.8853	11.000 / 0.0009	6.700 / 0.0096
SEX (N=55)				
	Male (n=26)	Female (n=6)	Male (n=13)	Female (n=10)
Genotypes				
Wild (E+/+)	24 (82.8%)	5 (17.2%)	9 (52.9%)	8 (47.1%)
Mutant / Wild (E+/-)	1 (50.0%)	1 (50.0%)	1 (100.0%)	0 (0.0%)
Mutant (E-/-)	1 (100.0%)	0 (0.0%)	3 (60.0%)	2 (40.0%)
Alleles				
Allele E+	49 (94.2%)	11 (91.7%)	19 (73.1%)	16 (80.0%)
Allele E-	3 (5.8%)	1 (8.3%)	7 (26.9%)	4 (20.0%)
HWE (X^2/p-value)	10.860 / 0.0010	0.050 / 0.8238	8.410 / 0.0037	10.000 / 0.0016
BMI (kg/m²) (N=53)				
	≤ 25 (n=20)	> 25 (n=11)	≤ 25 (n=8)	> 25 (n=14)
Genotypes				
Wild (E+/+)	19 (67.9%)	9 (32.1%)	7 (4102%)	10 (58.8%)
Mutant / Wild (E+/-)	1 (50.0%)	1 (50.0%)	0 (0.0%)	1 (100.0%)
Mutant (E-/-)	0 (0.0%)	1 (100.0%)	1 (25.0%)	3 (75.0%)
Alleles				
Allele E+	39 (97.5%)	19 (86.4%)	14 (87.5%)	21 (75.0%)
Allele E-	1 (2.5%)	3 (13.6%)	2 (12.5%)	7 (25.0%)
HWE (X^2/p-value)	0.013 / 0.9087	4.147 / 0,0417	8 / 0.0047	9.175 / 0.0025

BMI: Body mass index, HWE: Hardy Weinberg Equilibrium.

3.5. Presentation of the Population According to Serum Lipid and Apoprotein Levels

Regardless of HIV status, only the genotypic frequencies in subjects with high LDL-C were at HWE (Table 4). The mutant allele was more frequent in subjects with increased TG, LDL-C, Lp (a), atherogenicity index TC/HDL-C (Table 4) and Apo B100 (Table 5) and in those with normal total cholesterolemia, decreased HDL-C (Table 4) and Apo A1 (Table 5). Taking HIV status into account, the mutant allele was more frequent in PLHIV (14% to 32%) than in HIV- subjects (2% to 23%) (Table 6, Table 7). The mutant allele was more frequent especially in PLHIV with total hypercholesterolemia (28.1%), normal LDL cholesterolemia (26.7%), HDL hypocholesterolemia (27.3%), hyperapoproteinemia Lp (a) (28.1%), high atherogenicity index TC/HDL-C (23.7%) (Table 6), hypoapoproteinemia A1 (32.1%), hyperapoproteinemia B100 (28.1%) and high atherogenicity index ApoB/Apo A1 (23.7%) (Table 7).

Table 4. *Genotypes and alleles distribution according to lipid levels in the general population regardless of hiv infection status.*

PARAMETERS	**CONCENTRATION LEVEL**		
TG (g/L) (N=55)			
	Low (<0.3) (n=1)	Normal (0.3 – 1.2) (n=19)	High (>1.2) (n=35)
Genotypes			
Wild (E+/+)	1 (2.1%)	17 (37.0%)	28 (60.9%)
Mutant / Wild (E+/-)	0 (0.0%)	1 (33.3%)	2 (66.7%)
Mutant (E-/-)	0 (0.0%)	1 (33.3%)	5 (83.3%)
Alleles			
Allele E+	1 (100.0%)	35 (92.1%)	58 (82.9%)
Allele E-	0 (0.0%)	3 (7.9%)	12 (17.1%)
HWE (X^2/p-value)	NA	7.74 / 0.0054	22.35 / <0.0001
TC (g/L) (N=55)			
	Low (<1.06) (n=1)	Normal (1.06 – 2.5) (n=37)	High (>2.5) (n=17)
Genotypes			
Wild (E+/+)	1 (2.2%)	30 (65.2%)	15 (32.6%)
Mutant / Wild (E+/-)	0 (0.0%)	2 (66.7%)	1 (33.3%)
Mutant (E-/-)	0 (0.0%)	5 (83.3%)	1 (16.7%)

PARAMETERS	CONCENTRATION LEVEL		
Alleles			
Allele E+	2 (100.0%)	62 (83.8%)	31 (91.2%)
Allele E-	0 (0.0%)	12 (16.2%)	3 (8.8%)
HWE (X^2/p-value)	NA	23.74 / <0.0001	6.84 / 0.0089
LDL-C (g/L) (N=55)			
	Normal (<1.60) (n=41)		High (≥1.60) (n=14)
Genotypes			
Wild (E+/+)	35 (76.1%)		11 (23.9%)
Mutant / Wild (E+/-)	1 (33.3%)		2 (66.7%)
Mutant (E-/-)	5 (83.3%)		1 (16.7%)
Alleles			
Allele E+	71 (86.6%)		24 (85.7%)
Allele E-	11 (13.4%)		4 (14.3%)
HWE (X^2/p-value)	32.84 / <0.0001		2.43 / 0.119
HDL-C (g/L) (N=55)			
	Low (<0.40) (n=20)		Normal (≥0.40) (n=35)
Genotypes			
Wild (E+/+)	15 (32.6%)		31 (67.4%)
Mutant / Wild (E+/-)	1 (33.3%)		2 (66.7%)
Mutant (E-/-)	4 (66.7%)		2 (33.3%)
Alleles			
Allele E+	31 (77.5%)		64 (91.4%)
Allele E-	9 (22.5%)		6 (8.6%)
HWE (X^2/p-value)	14.68 / 0.0001		14.13 / 0.0002
Lipoprotéen Lp (a) (g/L) (N=55)			
	Normal (<0.25) (n=19)		High (≥0.25) (n=36)
Genotypes			
Wild (E+/+)	16 (34.8%)		30 (65.2%)
Mutant / Wild (E+/-)	1 (33.3%)		2 (66.7%)
Mutant (E-/-)	2 (33.3%)		4 (66.7%)
Alleles			
Allele E+	33 (86.8%)		62 (86.1%)
Allele E-	5 (13.2%)		10 (13.9%)
HWE (X^2/p-value)	11.26 / 0.0008		21.22 / <0.0001
Atherogenicity index 1: TC/HDL-C (N=55)			
	Normal (<3.3 [Women] or <4.4 [men]) (n=19)		High (≥3.3 [women] or ≥4.4 [men]) (n=36)
Genotypes			
Wild (E+/+)	17 (37.0%)		29 (63.0%)
Mutant / Wild (E+/-)	1 (33.3%)		2 (66.7%)
Mutant (E-/-)	1 (16.7%)		5 (83.3%)
Alleles			
Allele E+	35 (92.1%)		60 (83.3%)
Allele E-	3 (7.9%)		12 (16.7%)
HWE (X^2/p-value)	7.74 / 0.0054		23.04 / <0.0001

TG: Triglyceride, TC: Total cholesterol, LDL-C: low density lipoprotein-Cholesterol, HDL-C: high density lipoprotein-Cholesterol, HWE: Hardy Weinberg Equilibrium.

Table 5. *Genotypes and alleles distribution according to apoprotein levels in the general population regardless of HIV infection status.*

PARAMETERS	CONCENTRATION LEVEL		
Apo A1 (g/L) (N=55)			
	Low (<1.18) (n=21)	Normal (1.18 – 1.46) (n=19)	High (>1.46) (n=15)
Genotypes			
Wild (E+/+)	14 (30.4%)	19 (41.3%)	13 (28.3%)
Mutant / Wild (E+/-)	2 (66.7%)	0 (0.0%)	1 (33.3%)
Mutant (E-/-)	5 (83.3%)	0 (0.0%)	1 (16.7%)
Alleles			
Allele E+	30 (71.4%)	38 (100.0%)	27 (90.0%)
Allele E-	12 (28.6%)	0 (0.0%)	3 (10.0%)
HWE (X^2/p-value)	12.34 / 0.0004	NA	5.95 / 0.0147
Apo B (g/L) (N=55)			
	Low (<0.50) (n=2)	Normal (0.50 – 0.82) (n=9)	High (>0.82) (n=45)
Genotypes			
Wild (E+/+)	2 (4.3%)	7 (15.2%)	37 (80.5%)
Mutant / Wild (E+/-)	0 (0.0%)	1 (33.3%)	3 (66.7%)
Mutant (E-/-)	0 (0.0%)	1 (16.7%)	5 (83.3%)

PARAMETERS	CONCENTRATION LEVEL		
Alleles			
Allele E+	4 (100%)	15 (83,3%)	77 (85.6%)
Allele E-	0 (0%)	3 (16,7%)	13 (14.4%)
HWE (X^2/p-value)	NA	3,24 / 0,0719	24 / <0.0001
Atherogenicity index 2: ApoB/ApoA1 (N=55)			
	Normal (0.37 – 0.63) (n=11)		High (>0.63) (n=44)
Genotypes			
Wild (E+/+)	9 (19.6%)		37 (80.4%)
Mutant / Wild (E+/-)	1 (33.3%)		2 (66.7%)
Mutant (E-/-)	1 (16.7%)		5 (83.3%)
Alleles			
Allele E+	19 (86.4%)		76 (86.4%)
Allele E-	3 (13.6%)		12 (13.6%)
HWE (X^2/p-value)	4,15 / 0.0417		28.66 / <0.0001

HWE: Hardy Weinberg Equilibrium.

Table 6. *Genotypes and alleles distribution according to lipid levels and according to HIV infection status.*

PARAMETER	HIV- Subject			ART naive PLHIV		
TG (g/L) (N=55)						
	Low (<0.3) (n=1)	Normal (0.3 – 1.2) (n=12)	High (>1.2) (n=19)	Low (<0.3) (n=0)	Normal (0.3 – 1.2) (n=7)	High (>1.2) (n=16)
Genotypes						
Wild (E+/+)	1 (3.5%)	11 (37.9%)	17 (58.6%)	0 (0.0%)	6 (35.3%)	11 (64.7%)
Mutant / Wild (E+/-)	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)
Mutant (E-/-)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (20.0%)	4 (80.0%)
Alleles						
Allele E+	2 (100.0%)	23 (95.8%)	35 (92.1%)	0 (0.0%)	12 (85.7%)	23 (71.9%)
Allele E-	0 (0.0%)	1 (4.2%)	3 (7.9%)	0 (0.0%)	2 (14.3%)	9 (28.1%)
HWE (X^2/p-value)	NA	0.023 / 0.8803	7.74 / 0.0054	NA	7 / 0.0082	11.43 / 0.0007
TC (g/L) (N=55)						
	Low (<1.06) (n=1)	Normal (1.06 – 2.5) (n=19)	High (>2.5) (n=12)	Low (<1.06) (n=0)	Normal (1.06 – 2.5) (n=18)	High (>2.5) (n=5)
Genotypes						
Wild (E+/+)	1 (3.5%)	17 (58.6%)	11 (37.9%)	0 (0.0%)	13 (76.5%)	4 (23.5%)
Mutant / Wild (E+/-)	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)
Mutant (E-/-)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	4 (80.0%)	1 (20.0%)
Alleles						
Allele E+	2 (100.0%)	35 (92.1%)	23 (95.8%)	0 (0.0%)	27 (75.0%)	8 (80.0%)
Allele E-	0 (0.0%)	3 (7.9%)	1 (4.2%)	0 (0.0%)	9 (25.0%)	2 (20.0%)
HWE (X^2/p-value)	NA	7.74 / 0.0054	0.023 / 0.8803	NA	13.06 / 0.0003	5 / 0.0253
LDL-C (g/L) (N=55)						
	Normal (<1.60) (n=26)		High (≥1.60) (n=6)	Normal (<1.60) (n=15)		High (≥1.60) (n=8)
Genotypes						
Wild (E+/+)	24 (82.8%)		5 (17.2%)	11 (64.7%)		6 (35.3%)
Mutant / Wild (E+/-)	1 (50.0%)		1 (50.0%)	0 (0.0%)		1 (100.0%)
Mutant (E-/-)	1 (100.0%)		0 (0.0%)	4 (80.0%)		1 (20.0%)
Alleles						
Allele E+	49 (94.2%)		11 (91.7%)	22 (73.3%)		13 (81.2%)
Allele E-	3 (5.8%)		1 (8.3%)	8 (26.7%)		3 (18.8%)
HWE (X^2/p-value)	10.86 / 0.0010		0.050 / 0.8238	15 / 0.0001		2.78 / 0.095
HDL-C (g/L) (N=55)						
	Low (<0.40) (n=9)		Normal (≥0.40) (n=23)	Low (<0.40) (n=11)		Normal (≥0.40) (n=12)
Genotypes						
Wild (E+/+)	7 (24.1%)		22 (75.9%)	8 (47.1%)		9 (52.9%)
Mutant / Wild (E+/-)	1 (50.0%)		1 (50.0%)	0 (0.0%)		1 (100.0%)
Mutant (E-/-)	1 (100.0%)		0 (0.0%)	3 (60.0%)		2 (40.0%)
Alleles						
Allele E+	15 (83.3%)		45 (97.8%)	16 (72.7%)		19 (79.2%)
Allele E-	3 (16.7%)		1 (2.2%)	6 (27.3%)		5 (20.8%)
HWE (X^2/p-value)	3.24 / 0.0719		0.011 / 0.9151	11 / 0.0009		6.70 / 0.0096
Lipoprotéen Lp (a) (g/L) (N=55)						
	Normal (<0.25) (n=12)		High (≥0.25) (n=20)	Normal (<0.25) (n=7)		High (≥0.25) (n=16)
Génotypes						
Wild (E+/+)	10 (34.5%)		19 (65.5%)	6 (35.3%)		11 (64.7%)
Mutant / Wild (E+/-)	1 (50.0%)		1 (50.0%)	0 (0.0%)		1 (100.0%)
Mutant (E-/-)	1 (100.0%)		0 (0.0%)	1 (20.0%)		4 (80.%)

PARAMETER	HIV- Subject		ART naive PLHIV	
Alleles				
Allele E+	21 (87.5%)	39 (97.5%)	12 (85.7%)	23 (71.9%)
Allele E-	3 (12.5%)	1 (2.5%)	2 (14.3%)	9 (28.1%)
HWE (X^2/p-value)	4.60 / 0.0320	0,013 / 0,9087	7 / 0.0082	11.44 / 0.0007
Atherogenivity index 1: TC/HDL-C (N=55)				
	Normal (<3.3 [women] or <4.4 [men]) (n=15)	High (≥3.3 [women] or ≥4.4 [homme]) (n=17)	Normal (<3.3 [women] or <4.4 men]) (n=4)	High (≥3.3 [women] or ≥4.4 [men]) (n=19)
Genotypes				
Wild (E+/+)	14 (48.3%)	15 (51.7%)	3 (17.6%)	14 (82.4%)
Mutant / Wild (E+/-)	1 (50.0%)	1 (50.0%)	0 (0.0%)	1 (100.0%)
Mutant (E-/-)	0 (0.0%)	1 (100.0%)	1 (20.0%)	4 (80.0%)
Alleles				
Allele E+	29 (96.7%)	31 (91.2%)	6 (75.0%)	29 (76.3%)
Allele E-	1 (3.3%)	3 (8.8%)	2 (25.0%)	9 (23.7%)
HWE (X^2/p-value)	0.018 / 0.8938	6.84 / 0.0089	4 / 0.0455	13.87 / 0.0002

TG: Triglyceride, TC: Total cholesterol, LDL-C: low density lipoprotein-Cholesterol, HDL-C: high density lipoprotein-Cholesterol, HWE: Hardy Weinberg Equilibrium.

Table 7. *Genotypes and alleles distribution according to apoprotein levels and according to HIV infection status.*

PARAMETER	HIV- Subject			ART naive PLHIV		
Apo A1 (g/L) (N=55)						
	Low (<1.18) (n=7)	Normal (1.18 – 1.46) (n=11)	high (>1.46) (n=14)	Low (<1.18) (n=14)	Normal (1.18 – 1.46) (n=8)	High >1.46) (n=1)
Génotypes						
Genotypes	5 (17.2%)	11 (37.9%)	13 (44.8%)	9 (52.9%)	8 (47.1%)	0 (0.0%)
Wild (E+/+)	1 (50.0%)	0 (0.0%)	1 (50.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
Mutant / Wild (E+/-)	1 (100.0%)	0 (0%)	0 (0.0%)	4 (80.0%)	0 (0.0%)	1 (20.0%)
Mutant (E-/-)						
Alleles	11 (78.6%)	22 (100.0%)	27 (96.4%)	19 (67.9%)	16 (100.0%)	0 (0.0%)
Allele E+	3 (21.4%)	0 (0.0%)	1 (3.6%)	9 (32.1%)	0 (0/0%)	2 (100.0%)
Allele E-	2.32 / 0.1277	NA	0.019 / 0.8898	9.79 / 0.0018	NA	NA
Apo B (g/L) (N=55)						
	Low (<0.50) (n=1)	Normal (0.50 – 0.82) (n=3)	High (>0.82) (n=28)	Low (<0.50) (n=1)	Normal (0.50 – 0.82) (n=6)	High (>0.82) (n=16)
Genotypes						
Wild (E+/+)	1 (3.4%)	2 (6.9%)	26 (89.7%)	1 (5.9%)	5 (29.4%)	11 (64.7%)
Mutant / Wild (E+/-)	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)
Mutant (E-/-)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (20.0%)	4 (80.0%)
Alleles						
Allele E+	2 (100.0%)	5 (83.3%)	53 (94.6%)	2 (100.0%)	10 (83.3%)	23 (71.9%)
Allele E-	0 (0.0%)	1 (16.7%)	3 (5.4%)	0 (0.0%)	2 (16.7%)	9 (28.1%)
HWE (X^2/p-value)	NA	0.12 / 0.7290	11.75 / 0.0006	NA	6 / 0.0143	11.43 / 0.0007
Atherogenivity index 2: ApoB/ApoA1 (N=55)						
	Normal (0.37 – 0.63) (n=7)		High (>0.63) (n=25)	Normal (0.37 – 0.63) (n=4)		High (>0.63) (n=19)
Genotypes						
Wild (E+/+)	6 (20.7%)		23 (79.3%)	3 (17.6%)		14 (82.4%)
Mutant / Wild (E+/-)	1 (50.0%)		1 (50.0%)	0 (0.0%)		1 (100.0%)
Mutant (E-/-)	0 (0.0%)		1 (100.0%)	1 (20.0%)		4 (80.0%)
Alleles						
Allele E+	13 (92.9%)		47 (94.0%)	6 (75.0%)		29 (76.3%)
Allele E-	1 (7.1%)		3 (6.0%)	2 (25.0%)		9 (23.7%)
HWE (X^2/p-value)	0.041 / 0.8387		10.41 / 0.0013	4 / 0.0455		13.87 / 0.0002

HWE: Hardy Weinberg Equilibrium.

4. Discussion

This cross-sectional analytical study evaluated the impact of EcoR1 polymorphism on the occurrence of dyslipidemia during HIV1 infection not treated with antiretroviral drugs. It was carried out on 55 subjects including 23 ART-naive PLHIV (41.81%) and 32 HIV negative control subjects (58.18%).

High BMI was more common in PLHIV (63.6%) (*p=0.043%*) and so was the average BMI (*p=0.046*). In contrast, Muhammad et *al.* [19] as well as Ogunmola et *al.* [20] reported normal average BMIs (22.0 and 22.6 kg/m²) in

ART-naive PLHIV with lower proportions of overweight/obese subjects of 22% and 3% respectively. This difference could be explained by the dietary habits of the study populations or by the presence of predisposing factors in the subjects recruited in our study.

PLHIV showed some same lipid disorders as HIV- subjects, including hypertriglyceridemia, high Apo B and Lp (a) concentrations, and high atherogenicity indexes. However, PLHIV showed low Apo A1 concentrations ($p < 0.0001$) and HDL-C levels ($p = 0.016$). In their review of the literature, Green et *al.* [21] reported low serum TC, HDL-C and LDL-C concentrations and hypertriglyceridemia in ART-naive PLHIV. A study performed in Togo found higher proportions of PLHIV with hypercholesterolemia (41.4%) and lower proportions of subjects with hyper LDL-cholesterolemia (23.5%) and hypo HDL-cholesterolemia (17.4%) [2]. It has been found 64.4% hypo-HDL-cholesterolemia in untreated PLHIV [3]. Other authors found, contrary to us, hyperapoproteinemia A1 [10]. These differences could be explained by the features of the study populations. Indeed, Agbeko et *al.* explored lipid disorders in PLHIV on ART for at least 12 months [2] while untreated PLHIV constituted the population in our study and that of Appiah et *al.* [3] evaluated antiretroviral molecules influencing lipid level. The difference in nutritional habits of the study populations could explain these discrepancies because lipid status is correlated with diet. Genetic predisposition factors, which themselves vary according to the geographical location of the subjects, could contribute to this variability. These differences in profiles could also be due to the stages of immunodepression of the patients. Indeed, it has been shown that HIV infection induces an early decrease in cholesterol generally affecting TC first, followed by HDL-C and then LDL-C, and a late increase in TG and these changes are correlated with the degree of immunosuppression in a number of cases [22].

All 3 possible genotypes of EcoR1 polymorphism were found. In contrast, Kone et *al.* (2018) in Côte d'Ivoire [12] and Sharma et *al.* in India [11] did not find the mutant genotype (E-/-) in their studies. This could be attributed to the size of the population but also to the geographical location of the population. The HWE was deviated in both HIV- subjects ($X^2 = 6.97$; $p = 0.008$) and PLHIV ($X^2 = 19.20$; $p < 0.0001$). This result is consistent with that of Kodogo et *al.* [23]. The mutant genotype was more common in PLHIV (85.7%). The frequency of the mutant allele (E-) was 27.1% and significantly higher in PLHIV. Kodogo et *al.* found only 1.2% of PLHIV with the mutant genotype and a lower frequency of mutant allele (E-) (15%) [23].

The results observed in the general population differed from those observed in each group. Thus, the division into PLHIV and HIV- subjects allowed us to clarify the impact of the EcoR1 polymorphism in PLHIV. Mutant and heterozygous genotypes as well as E- allele (20%) predominated in subjects with high BMI. The presence of E- allele appeared to be associated with the occurrence of obesity/overweight. In a study made in India [24] and an other in Singapore [25] found no association between EcoR1 polymorphism and obesity.

The mutant allele was more frequent especially in PLHIV with total hypercholesterolemia (28.1%), normal LDL cholesterol (26.7%), HDL hypocholesterolemia (27.3%), hypoapoproteinemia A1 (32.1%), hyperapoproteinemia B100 (28.1%), hyper Lp (a) (28.1%), and high atherogenicity indices (23.7%). Kone et *al.* [13] showed in 2017 that mutations in the Apo B100 gene were more common in PLHIV. Furthermore, the association between the EcoR1 polymorphism and serum lipid variations depends on the lipid and the study population. As a matter of fact, regardless of the subject genotype, the majority of subjects, both healthy and PLHIV showed hypertriglyceridemia, normal TC concentration, high Apo B and Lp (a) values, and high atherogenicity index. The EcoR1 polymorphism did not appear to have an impact on these parameters. In their study, Sharma et *al.* showed hyperApo B only in E+/- subjects [11]. For Gu W et *al.*, serum TC was significantly higher in subjects carrying E- allele [26]. For Hu P et *al.*, subjects with the homozygous mutant genotype (E-/-) showed high serum Lp (a) and TC concentrations [9].

Serum LDL-cholesterolemia were high in heterozygotes (E+/-). However, since HWE is respected, the carrying of E- allele would not expose the subject to high LDL-C concentration. This result was in contrast to that found in a Chinese study which showed that the mutant allele was correlated with high LDL concentrations [26]. However, low HDL-C were found in mutants. Hypoapoproteinemia A1 was predominant in mutant and heterozygous genotypes. The carrying of E- allele would promote a decrease in serum Apo A1 responsible for the low HDL-C concentrations in E-/- homozygotes; also found by Gu W et *al.* [26]. In contrast, Hu P's team [9] reported HDL hypercholesterolemia and hyperApoproteinemia A1 in E-/- homozygotes. These results change from one study to another and this discordance may be induced by the variability due to the study population (age, origin, etc.) but also to the sample size. The interaction of these factors with other genetic, environmental or nutritional factors could alter the effect of the polymorphism [11, 12, 27].

5. Conclusion

The results observed in the general population differed from those in PLHIV. The distribution of alleles was not random, and mutant allele (E-) was more frequent in PLHIV and exposed them to high BMI, increased atherogenic apolipoproteins, atherogenicity index and decreased protective apolipoproteins. In order to investigate the link between the effect of ART and Apoproteins B100 and A1 genes polymorphisms, we are considering doing analyses on a population of PLHIV on ART.

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