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rs1445776009 variants in the human *ALB* gene: Association with serum albumin and clinical outcomes in HIV-infected Kenyan injection substance users

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ABSTRACT

Objective: The coburden of human immunodeficiency virus (HIV) and injection substance use is high especially in coastal urban and peri-urban regions of sub-Saharan Africa. Although antiretroviral treatment (ART) has improved disease prognosis in HIV-1-infected patients, injection substance use is detrimental to these individuals. HIV-1 and injection substances use have been associated with a marked reduction in serum albumin levels. This is attributable to at least, in part, injection substance use, ARVs, HIV-1 infection, as well as host genetics. The albumin gene expression is modulated through several mechanisms including intronic consensus elements. Therefore, this study examined *ALB* gene rs1445776009 intronic polymorphism and its association with disease outcomes.

Methods: This cross-sectional study was conducted at Bomu Hospital, Mombasa County, Kenya. A total of 155 injection substance users (ISUs) were recruited comprising 93 ART experienced and 62 ART naive. Variant rs1445776009 was amplified through polymerase chain reaction and genotyped through restriction fragment length polymorphism.

Results: Carriers of the mutant and GG had significantly lower body mass index ($P = 0.033$), serum albumin ($P = 0.002$), CD4+ T cells ($P = 0.031$), and higher HIV-1 RNA copies ($P = 0.018$) relative to wild type, CC and heterozygous, CG; in ART-experienced ISUs. In addition, mutant GG carriers were at higher odds of presenting with hypoalbuminemia (OR, 1.933; 95% CI, 1.524–4.664; $P = 0.033$), underweight (OR, 2.412; 95% CI, 1.124–5.782; $P = 0.026$), immunosuppression (OR, 3.036; 95% CI, 1.957–9.633; $P = 0.021$), and high-density HIV viremia (OR, 1.836; 95% CI, 1.134–6.298; $P = 0.016$).

Conclusion: This study's findings appear to suggest that loci rs1445776009 of the *ALB* gene could be modulating serum albumin levels and disease outcomes in HIV-1 ART-experienced ISUs.

Keywords: *ALB* gene, antiretroviral treatment, human immunodeficiency virus-1, injection substance user, serum albumin

Introduction

Injection substance use and human immunodeficiency virus (HIV) are global public health problems.[1] In sub-Saharan Africa, injection substance use is on the increase, especially among youths in urban and peri-urban centers.[2] In Kenya, Mombasa County in the coastal region has reported the highest rate (19%) of HIV infection among injection substance users (ISUs).[3] The increase in HIV infections and rapid progression of the disease can be attributed to substance use.[3] Introduction of antiretroviral treatment (ART) has greatly improved prognosis among HIV-infected individuals and reduced transmission rates in the population.[4] Nevertheless, injection substance use among HIV-infected individuals often dissuades the benefits of ART.[5] This largely due to poor adherence to treatment protocols including the complex molecular interaction of injection substances and ART.

Human albumin is an abundant vital plasma protein synthesized in the liver.[6] It mainly regulates the oncotic pressure and maintains acid base balance.[7] In addition, albumin is a key transport protein for cations, fatty acids, and xenobiotics including pharmaceutical and recreational drugs.[8] Albumin has also been shown to have antioxidant and anti-

inflammatory properties, hence protective against deleterious xenobiotic effects.[9] Furthermore, liver, renal functions, and nutritional status are monitored using serum albumin as a clinical marks.[10,11] HIV infection has been associated with reduced serum albumin levels in both ART-naive and -experienced individuals.[12,13] Similarly, substances such as heroin, cocaine, bhang, and alcohol have also been shown to decrease serum albumin levels.[14] Furthermore, our previous studies and those of others have also substantiated that HIV-1 infection and substance use influence serum albumin levels.[2,12]

Serum albumin levels have been associated with markers of disease progression in HIV-1-infected individuals.[15] For instance, decreased serum albumin has been linked with underweight,[11] immunosuppression,[16] and high-density HIV viremia.[17] Altogether, these findings suggest that low circulating albumin levels, at least, partly, result from HIV-induced hepatopathy effect. In addition, prolonged use of ARTs and substances causes hepatotoxicity and severe liver downregulation.[18] These, in turn, lead to reduced liver synthetic function as a result of stimulating effects of psychoactive substances.[14,19] This complex interaction of these molecules with host genetics impacts negatively on management of HIV-1 infection in the ART-experienced ISUs.

Human albumin is encoded by the *ALB* gene.[7] The expression of *ALB* gene is modulated by its promoter, transcription factors, and introns.[20] The role of intronic variations in modulating the *ALB* gene expression has been demonstrated. For instance, in a German, infant having (c.270+1G>T) mutation in intron III was associated with severely depressed serum albumin levels (4). However, the role of rs1445776009 intron VII variant in regulating *ALB* gene expression has not been reported. It is likely that intronic variations influence the *ALB* gene expression through intronic mRNA sequence splicing and forming sites for binding of splicing proteins such as hnRNP protein.[21] In as much as introns are spliced off mRNA, several studies have revealed their key roles in gene expression.

For instance, intron VII of the *ALB* promotes gene expression through enhancing mRNA transport, chromatin assembly, and splicing.[22] In addition, proteolytic processing of the pro-albumin generates EPI-X4 antagonist of CXCR4 during post-translation, a key component of the immune system.[23] Likewise, intron VII is a key component in the alternative splicing of the gene.[24] Several mutations in the *ALB* gene are associated with serum albumin levels.[25] For instance, novel intron III (c.270+1G>T) and intron II (c.138-2 A>G) have, respectively, been associated with serum albumin levels.[4,26] These findings appear to imply that the *ALB* gene intronic variants influence serum albumin levels.

HIV-1-infected ISUs have a complex interaction of multiple factors that could influence albumin levels and disease outcomes. As such, these individuals are at risk of suffering adverse effects. However, limited information exists on the complex interaction of host genetics, HIV-1, substance, and ART use on disease outcomes and serum albumin levels. Therefore, this study examined the association of *ALB* intron VII rs1445776009 genotypic variation with circulating albumin and clinical markers of HIV infection in Kenyan ISUs.

Materials and Methods

Study design, area, and population

This cross-sectional study was conducted as part of a wider study on HIV infection among substance users from July 2012 to February 2013 in Mombasa County, a Coastal city in Kenya. The County has a large injection substance consuming inhabitants of about 37,000, with heroin being the most prevalent injection drug.[27] Injections substance users were enrolled into the study through respondent driven sampling, snowball, and makeshift outreach methods. Only individuals (age ≥18 years) exhibiting needle scars, reporting injection heroin use at least once in the previous month, and providing written informed consent were recruited into the study.[12] Briefly, the current genotyping study analyzed ART-experienced (n = 93) and ART-naive (n = 62) HIV-1-positive ISUs. Participants presenting clinically with other conditions other than HIV-1 and drug use were excluded from the study. Participants with a history of liver, kidney, and cardiovascular diseases including chronic diseases were excluded from the study. All the ART-experienced study participants were on first-line treatment comprising tenofovir disoproxil fumarate (TDF) + lamivudine (3TC) + efavirenz (EFV), as well as cotrimoxazole prophylaxis.[28] The sample size was calculated using Wayne (2016) formula [ni = {p1 (1- p1) +p2 (1- p2)} (Z/E) 2], based on previously reported genotype frequencies of wild type and mutant rs1445776009 SNPs in the *ALB* gene among a sub-Saharan Africa Bantoid ethnic group from South Africa.[29] In this formulae, p1 (wild-type) = 0.64 was entered as p1 (mutant); p2 = 0.36 as p2, Z =1.96 as Z (standard normal variants for the chosen [confidence level 95%]) and E = 0.2 as E (margin of allowable error). The value obtained was minimum required for cases, therefore was matched with controls.

Demographic and Clinical Information

A standard questionnaire with open-ended questions was used for collecting demographic and substance use information as previously described in our previous studies.[12,30] Anthropometric data (weight, kg; and height, m) were collected using mechanical weighing and height scale (RGZ-160) machine and body mass index (body mass index [BMI], kg/m^2) was calculated and classified based on the World Health Organization adult nutritional status[31] into underweight (BMI <18.5 kg/m^2), normal body weight (BMI ≥18.5 < 25.0 kg/m^2), and overweight BMI ≥25.0 kg/m^2). HIV status was determined according to protocols of the Kenya National HIV Testing and Counseling Guidelines (NASCOP, 2010).

CD4+ T-cell accounts

Baseline CD4+ T-cell enumeration was determined in using the BD FACSCalibur flow cytometer (Becton-Dickinson™, Franklin Lakes, USA). Briefly, 5.0 µl of EDTA blood specimen were placed in a tube and RBC lysis buffer added. After 5 min incubation, the cells were washed and fluorescent-tagged antibodies (anti-CD3, anti-CD4, and anti-CD45) were added. The cells were incubated for 30 min after which the samples were washed and the CD4+ T cells enumerated on the flow cytometer. The baseline CD4+ T-cell counts were defined based on the WHO guidelines, immunosuppression <CD4+ T cells/µl.[28]

HIV-1 viral load

HIV-1 viral loads were determined through the automated Abbott m2000 System following the manufacturer's protocols (Abbott Molecular Inc., Illinois, USA). Briefly, RNA was extracted from 0.2 ml serum samples and reverse transcribed into cDNA. The cDNA was amplified using HIV-1 specific and internal control primers. Fluorescence intensity of the HIV-1 probe was converted into viral loads by the analyzer. The baseline viral load was defined based on the WHO guidelines, viral suppression was defied as <1000 HIV-1 RNA copies/ml.[28]

DNA extraction and rs1445776009 genotyping

DNA was extracted from dried blood spots (Whatman™ FTA™ Cards) using QiaAmp™ DNA Mini Kit (Qiagen Inc., Valencia, USA) and genotyping performed as previously described.[32] Human *ALB* gene at intron VII loci 73409488-73410312 was amplified by nested polymerase chain reaction (PCR) using Gene Amp™ PCR system 9700 (Applied Biosystems, Foster County, USA). PCR reactions were performed in a total volume of 50 µL containing 25 µL of PCR master mix which constituted 5 µL of PCR buffer, 1 µL of dNTPs (10 mM), 1 µL of forward primer (50 pmol), 1 µL of reverse primer (50 pmol), 1 µL Taq DNA polymerase (5 U/µL), 5 µL of template DNA, 1 µL of $MgCl_2$ (50 mM), and 10 µL of deionized water, 1 µL of forward primer (50 pmol), 1 µL of reverse primer (50 pmol), 5 µL of template DNA, and finally 18 µL of deionized water. The primer sequences used were primer 1 (sense), 5'-GTAGGTGGACTTGGAGAAGG-3' (nucleotides 9477-9496), and primer 2 (antisense), 5'-GATATACTTGGCAAGGTCCG-3' (nucleotides 9132-9151). PCR amplification conditions included a denaturing cycle at 94°C for 10 min, followed by 30 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR amplified product was digested in a total volume 50 µL which comprised 10 µL (DNA), 1 µL of Hae III enzyme, and 5 µL of 10 × NEB buffer and 34 µL of deionized water at 37°C overnight. The products of digest were separated using ethidium bromide stained 1% agarose gel electrophoresis at 120 volts and the bands visualized under ultraviolet light.

Serum albumin levels

The baseline serum albumin levels were determined using automated clinical chemistry analyzer according to the manufacturer's instructions and reported in g/l (Roche Cobas 6001 (Roche COBAS® 6001, Lausanne, Switzerland).[33] Briefly, the machine work on the cationic features, albumin displays at acidic pH (citrate buffer: 95 mmol/L, pH 4.1) that enables it binds bromocresol green (0.075 M) (an anionic dye), at absorbance of 628 nm and room temperature with composition of NaCl 9%, 50 mL as a salt to form a blue-green complex. The intensity of the color is directly proportional to the albumin concentration in the sample that is determined photometrically. The hypoalbuminemia was defined based on previous established normal reference range of clinical biochemical parameter among normal Kenyan adults at Kenyatta National and Teaching and Referral Hospital (male <29–52 g/l and female 28–50 g/l).[34]

Statistical analyses

Data analyses were conducted using IBM SPSS version 24 (IBM SPSS Inc., New York, USA). Proportions of gender, viral suppression (HIV RNA <1000 copies/ml), immunosuppression (CD4+ T cells<500 cells/ml), underweight (BMI <18.5 kg/m^2), and rs1445776009 genotypes were compared between the cases and controls using Fisher's exact test. Variation in the genotypic and allelic frequencies was calculated using the Hardy Weinberg equilibrium online tool at http://ihg.helmholtz-muenchen.de/ihg/snps.html. Age, height, weight, BMI, CD4+ T cells, HIV RNA copies, and serum albumin levels were compared between the cases and controls using the Mann–Whitney U-test, and subsequently across the rs1445776009 genotypes using the Kruskal–Wallis test with Dunn's *post hoc* correction for multiple comparisons. Multivariate logistic regression was used to determine the association between genotypes and clinical markers of HIV infection, as well as with serum albumin levels in the cases and controls. All tests were two tailed and $P < 0.5$ was considered statistically significant.

Ethical considerations

The study was conducted according to the Helsinki Declarations,[35] with ethical approvals obtained from the MMUST IREC (Protocol: MMU/COR: 403012 vol2 (17)) and Kenyatta University ERC (Protocol: PKU019/116/2012). Informed consent was obtained from all the study participants before enrolment into the study and confidentiality was observed throughout the study.

Results

Demographic and laboratory characteristic of the study participants

The demographic and laboratory characteristic of the study participants is presented in Table 1. Of the 155 ISUs recruited

into this study, (n = 93) were HIV-1 infected ART-experienced and (n = 62) ART-naive individuals. The gender (P = 0.262), age (P = 0.685), and height (P = 0.387) were similar between ART-experienced and -naive individuals. However, ART-experienced ISUs had significantly lower weight (median 53.0 IQR 6.5 kg/m^2 vs. median 54.0 IQR 8.3 kg/m^2; P = 0.045) and BMI (median 18.3 IQR 2.18.3 kg/m^2 vs. median 18.8 IQR 2.48.3 kg/m^2; P = 0.029) relative to ART-naive ISUs. Consistent with low median of weight and BMI, the rates of underweight were significantly higher in the ART-experienced (53.8) compared to ART-naive (32.3; P = 0.008) individuals. Laboratory evaluation indicated that serum albumin levels were significantly lower in the ART-experienced (median 27.5 IQR 11.2 g/l) relative to ART-naive (median 33.5 IQR 8.5 g/l; P = 0.003) ISUs. In addition, the rates of albumin suppression, hypoalbuminemia, were high 39 (61.9) in the ART-experienced individuals in comparison to ART-naive 9 (30.0; P = 0.004) individuals. Evaluation of immune status showed that the rate of CD4 T-cell count was significantly low in the ART-experienced (median 392 IQR 334 cell/) compared to ART-naive (median 518 IQR 468 cell/; P = 0.022) ISUs. Consistent with lower CD4 T-cell count, the ART-experienced had significantly higher rates of immunosuppression 62 (66.7) compared to the ART-naive 31 (50.0: P = 0.038) individuals. Consistent with immunosuppression, pattern of HIV-1 RNA levels was significantly high in ART-experienced (median 2.6 IQR 2.3 copies/ml) compared to ART-naive (median 2.2 IQR 2.1 copies/ml; P = 0.036) individuals. Besides, high levels of HIV-1 viremia, the rates of high-density HIV viremia were significantly high in ART experienced 53 (58.2) as compared to ART naive 30 (49.2; P = 0.042).

Table1: Demographic and laboratory characteristics of the study participants

Characteristic	HIV-1[+]/ART[-] ISUs, n=62	HIV-1[+]/ART[+] ISUs, n=93	P
Female	31(50.0)	55(59.1)	0.262
Age, years	30.6(6.4)	30.6(6.6)	0.685
Height, m	1.7(0.1)	1.7(0.1)	0.387
Weight, kg	54.0(8.3)	53.0(6.5)	0.045
BMI, kg/m^2	18.8(2.4)	18.3(2.1)	0.029
Underweight	20(32.3)	50(53.8)	0.008
[a]Albumin, g/l	33.5(8.5)	27.5(11.2)	0.003
Hypoalbuminemia	9(30.0)	39(61.9)	0.004
CD4+T cells/µl	518(468)	392(334)	0.022
Immunosuppression	31(50.0)	62(66.7)	0.038
Log^{10} HIV-1 RNA, copies/ml	2.2(2.1)	2.6(2.3)	0.036
High-density HIV-1 viremia	30(49.2)	53(58.2)	0.042

Data are presented as number(n) and proportion(%) of subjects for gender or as median and interquartile range(IQR) for age, height, weight, BMI: Body mass index, albumin, CD4+T cells, and log10 HIV-1 RNA copies. HIV-1[+]: Human immunodeficiency virus-1 positive. ART: Antiretroviral treatment experienced[+] and naive[−]. ISUs: Injection substance users. BMI<18.5 kg/m^2, underweight. Serum albumin levels<29.0 g/l(males) and<28.0 g/l(females), hypoalbuminemia. CD+T cells<500/-l, immunosuppression. Log10 HIV-1 RNA copies≥1000 copies/ml, high-density HIV-1 viremia. [a]Albumin levels were measured in a subset of both ART-experienced(n=62) and-naive(n=30) individuals. Data analysis was performed using the Mann–Whitney U-tests for continuous variables and Fisher's exact tests for categorical variables. Bolded values indicate significant P-values

Human ALB gene intron VII rs1445776009 loci genotypes and alleles frequency in the ISUs are presented in Table 2. The carriage of wild-type CC genotype was high in the overall study population 78 (50.3) as well as in the ART experienced 41 (44.1) and naive 37 (59.7). The carriage of heterozygous state, however, was lower both in the overall study population 37 (23.9) as well as in the ART-experienced 25 (26.9) and -naive 12 (19.3) study participants. The rate of mutant GG genotype carrier was also similar across overall population 40 (25.8) and the two study groups ART experienced 27 (29.0) and naive 13 (21.0). Chi-square analysis confirmed that this genotypes distribution was similar between study groups (P = 0.074). Likewise, the allele distribution was similar across the overall population, C (major) 193 (62.3), G (minor) 117 (37.7) and ART-experienced C (major) 107 (57.5), G (minor) 79 (42.5) and -naive C (major) 86 (69.4), G (minor) 38 (30.6) individuals. Further, genetic Hardy–Weinberg analysis indicated that allele frequencies was similar in the overall population (P = 9.03 $^{-10}$) as well as in the ART experienced (P = 0.000014) and naive (P = 0.000018). In addition to testing allele frequency difference and the increase risk of homozygous carriers, we also used additive recessive and dominant models of inheritance to compare the genotype frequency between controls and cases as presented in Table 3.

HIV-1 infection syndrome in rs1445776009 genotypes is presented in Figure 1. Among the ART-experienced individual, BMI differed significantly across the genotypes, CC (median 18.8 IQR 1.9 kg/m^2), CG (median 18.7 IQR 2.2 kg/m^2), and GG (median 17.7 IQR 1.3 kg/m^2); P = 0.033. Likewise, serum albumin levels differed among genotypes (P = 0.002), with GG (median 20.0 IQR 8.3 g/l) and CG (median 29.6 IQR13.3 g/l) carrier having lower levels than CC (median 30.0 IQR 9.5 g/l) genotype in experienced individuals. In addition, CD4 + T-cell count was lower in GG (median 311 IQR 264 cell/) and CG (median 313 IQR 426 cell/ as compared to CC (median 473 IQR 554 cell/; P = 0.031) genotype in

Table2: Human ALB gene intron VII rs1445776009 loci genotype and allele distribution in the injection substance users

Genetic parameters	HIV-[+]/ ART[−], n=62	HIV-1[+]/ ART[+], n=93	P	All patients
Genotype				
CC	37(59.7)	41(44.1)	0.074	78(50.3)
CG	12(19.3)	25(26.9)		37(23.9)
GG	13(21.0)	27(29.0)		40(25.8)
Alleles				
C	86(69.4)	107(57.5)		193(62.3)
G	38(30.6)	79(42.5)		117(37.7)
χ^2 HWE	χ^2=18.39 P=0.000018	χ^2=18.32 P=0.000014		χ^2=37.53 P=9.03^-10

Data presented are numbers(n) and proportions(%) of subjects. HIV-1[+]: Human immunodeficiency virus type-1 positive. ART: Antiretroviral treatment experienced[+] and naive[−]. ISUs: Injection substance users. CC: Homozygous wild type; CG: Heterozygous and GG: Homozygous mutants. C: Major allele and G: Minor allele. HWE: Hardy–Weinberg equilibrium. Genotype distribution between the ART-experienced and-naive individuals was compared using the Pearson's Chi-square test. Values in bold are significant P-values

Table 3: Genotype and allele relative risk between ART-naive and-experienced study participants.

Statistical parameter	Allele positivity				Armitage trend
	Allele frequency	Homozygous risk	Dominant model	Recessive model	Additive model
	[C] versus[G]	[CC+] versus[GG]	[CC] versus[CG+GG]	[GG] versus[CG+CC]	G common odds
O.R	1.671	1.880	1.874	1.877	1.388
C.I. 95%	1.034–2.700	0.829–4.266	0.845–4.159	0.978–5.605	
χ^2	4.43	2.31	2.42	3.62	2.97
P	0.0353	0.1285	0.1201	0.0572	0.0849

Data presented are odds ratio(OR), C.I. 95%, confidence interval at 95%, χ^2: Chi-square, CC: Homozygous wild type; CG: Heterozygous and GG: Homozygous mutants. C: Major allele and G: Minor allele. The letter and value in bold are significant *P*-values, allele and genotypes for which the risk was calculated

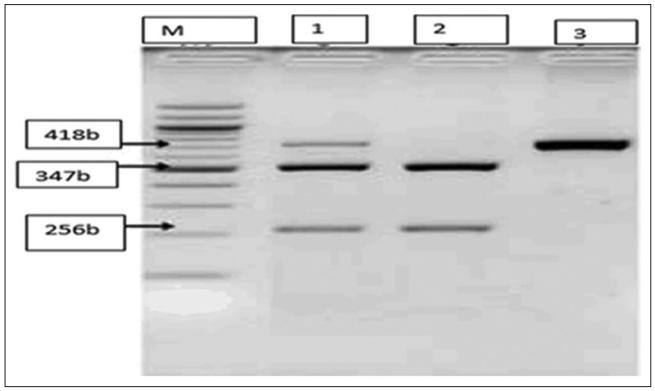

Figure 1: Gel electrophoresis of the Hae III digest of the polymerase chain reaction (PCR) amplicons. The figure shows restriction fragment length polymorphism analysis of the *Hae III* digest of the PCR amplicons that targeting locus rs1445776009 of the *ALB* gene separated on a 2% agarose gel. Lane M: DNA marker uncleaved PCR product of size 418 bp, (1) heterozygous (CG) shows three bands of sizes 418, 347, and 256 bp, (2) homozygous mutant (GG) shows two bands of sizes 347 and 256 bp, and (3) homozygous wild type (CC) shows one band of 418 bp

the experienced individuals. In contrast, HIV-1 viremia was significantly different across the genotype $P = 0.018$, with the GG (median 3.5 IQR 2.2 copies/ml) and CG (median 2.3 IQR 1.6 copies/ml) having higher levels of HIV-1 viremia as compared to CC (median 2.2 IQR 2.2 copies/ml) genotypes. Figure 2 shows the serum albumin, BMI, CD4+ T cells, and human immunodeficiency virus (HIV)-1 RNA levels in ALB gene intron VII rs1445776009 genotypes among antiretroviral treatment (ART)-experienced and -naive individuals.

Frequency and association of underweight, hypoalbuminemia, immunosuppression, and high-density HIV-1 viremia with human ALB gene intron VII rs1445776009 genotypes with in ART- experienced and -naive individuals, [Figure 3]. The rates of underweight were higher in the mutant and GG carrier (77.8) among the ART-experienced individuals ($P = 0.011$) but not in the ART-naive (46.2) individuals ($P = 0.091$). Likewise, GG carrier among ART experienced had high rates of hypoalbuminemia (85.7; $P = 0.022$) but not in the ART-naive (42.8; $P = 0.082$) individuals. Similar pattern was observed for rates of immunosuppression and high-density HIV viremia in the GG carrier among ART-experienced (88.9; $P = 0.010$) and (85.9; $P = 0.020$) but not in the ART-naive (53.8; $P = 0.309$) and (53.8; $P = 0.721$) ISUs, respectively. Binary logistic modeling in the experienced indicated that mutant, GG in relation to wild-type CC, genotype associated with underweight BMI (OR, 2.412; 95% CI, 1.21–5.78; $P = 0.026$), hypoalbuminemia (OR, 1.874; 95% CI, 1.32–4.5.81; $P = 0.008$), immunosuppression (OR, 3.036; 95% CI, 1.96–9.63; $P = 0.023$), and high-density HIV-1 viremia, (OR, 1.836; 95% CI, 1.13–1.29; $P = 0.012$). In addition, the CG genotype was associated with underweight but not with other HIV syndromes, $P < 0.05$.

Discussion

Serum albumin is a key protein that is encoded by albumin gene in the liver.[6] It performs a myriad of physiological roles in the body, transportation of xenobiotic compounds to the liver, and other tissues for metabolism, drugs, and substance use included.[7] The expression of the gene is regulated in both promoter, exon, exon-intron, and intron sites during transcription, translation, and post-translational modification process.[23,36] Taken together, quantity and structure of serum albumin in long ran affect binding of compounds. Studies have reported markedly reduced serum albumin levels in the ISU, HIV-1 infected, as well as ART-experienced individuals.[14,15] This could imply that reduced serum albumin level could be as a result of substance use, HIV-1 infection, and ART. Furthermore, host genetics has also been implicated to have an influence on gene.[4]

The *ALB* gene variant rs144577009 has not been previously shown to modulate HIV-1. In addition, no previous evidence has associated variation at rs144577009 with infectious diseases. However, genotyping revealed that wild-type, CC genotype carriage, and C allele were prevalent in both ART-experienced, -naive, and in the general ISUs study population. Conversely, heterozygous CG genotypes carriage was lowest in both ART-naive and -experienced ISUs. In addition, the allele frequency was similar and consistent with the Hardy–Weinberg equilibrium. These findings imply that it's an admixed population with low heterozygosity. Although, the sample size was not large enough to affirm low heterozygosity. Low heterozygosity suggests consanguinity in a population. This finding agrees with our previous report on the ADIPOQ

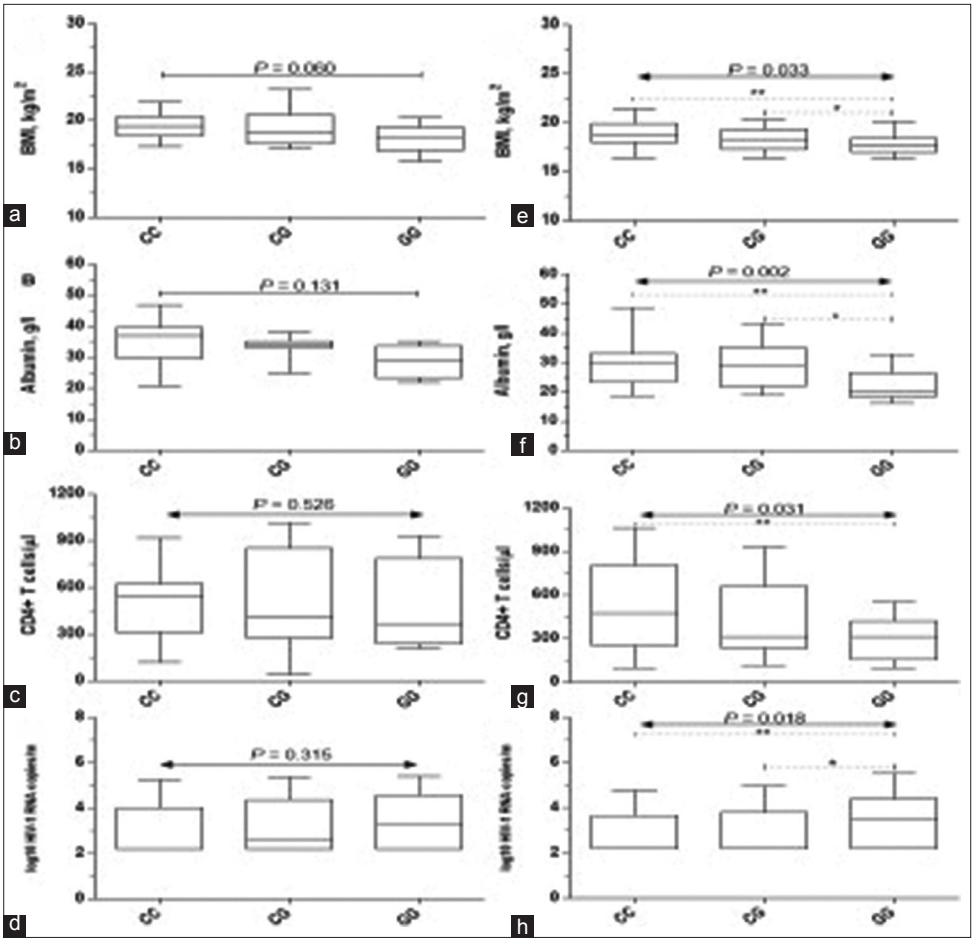

Figure 2: Serum albumin, BMI, CD4+ T cells, and human immunodeficiency virus (HIV)-1 RNA levels in *ALB* gene intron VII rs1445776009 genotypes among antiretroviral treatment (ART)-experienced and -naive individuals. Data shown are box plots with the box representing 25th–75th percentiles and the line through the box indicating the median, while whiskers show 10th and 90th percentiles. (a) Body mass index (BMI), (b) serum albumin, (c) CD4+ T cells, and (d) Log_{10} HIV-1 RNA levels in ART-naive individuals. (e) Body mass index (BMI), (f) serum albumin, (g) CD4+ T cells, and (h) Log_{10} HIV-1 RNA levels in ART-experienced individuals. CC, homozygous wild type; CG, heterozygous and GG, homozygous mutants. Data analyses were performed using Kruskal–Wallis tests followed by Dunn's *post hoc* corrections for multiple comparisons for significant Kruskal–Wallis tests. **P*<0.05 and ***P*<0.01 versus CG heterozygotes

gene showing low heterozygosity.[37] Further, incidence of incest and marriage between relatives has been reported in this community, which promotes inbreeding.[38]

Clinical markers of HIV-1 infection, BMI, serum albumin levels, CD4+ T cells, and HIV-1 RNA copies were significantly lower in the mutants, GG genotypes carriage relative to the wild-type CC, genotype carriage in the experienced individuals, suggesting that the GG genotype is associated with marked HIV-1 disease. Mutant GG genotype at loci rs144577009 was found to be associated with adverse disease outcomes in HIV-1 ART participants. The current study cannot link selection of mutant genotypes to HIV status. Instead, this finding could suggest that having the mutant genotype at these loci may influence *ALB* gene expression. Subsequently, this could lower the circulating levels of serum albumin and, in turn, influence clinical outcomes. Previous non-genetic studies have reported low BMI and serum albumin in HIV-1-infected individuals.[11,39] Similarly, ISU has reported low BMI and reduced serum albumin levels.[14,40,41] Further, chronic ART use has reported similar trend in HIV-1-infected individuals.[42,43] Consistent with the findings, low CD4+ T cells count and high-density HIV-1 RNA copies have been reported in the HIV-1-infected ART-experienced ISUs.[13,16] In contrary to the finding, others studies have reported increase in BMI, serum albumin levels, CD4+ T cells, and reduced in HIV-1 RNA copies in Phase 1 of ART-experienced individuals.[44,45] Although, this is the first study, to the best of our knowledge, associating intronic variation in *ALB* with low serum albumin in HIV-1 ISUs. Low BMI among ART-experienced ISUs could be attributed to unexplained chronic diarrheal, HIV enteropathy, and malabsorption which are high among in individuals on chronic ARTs use.[43,46] Conversely, reduced serum albumin in the ART-experienced individuals could be attributable to the high rates of breakdown of albumin due to inflammation and deteriorating of the nutritional status in ART using HIV-infected non-substance users.[47] In addition, the low serum albumin levels are due to the hepatotoxicity effects of

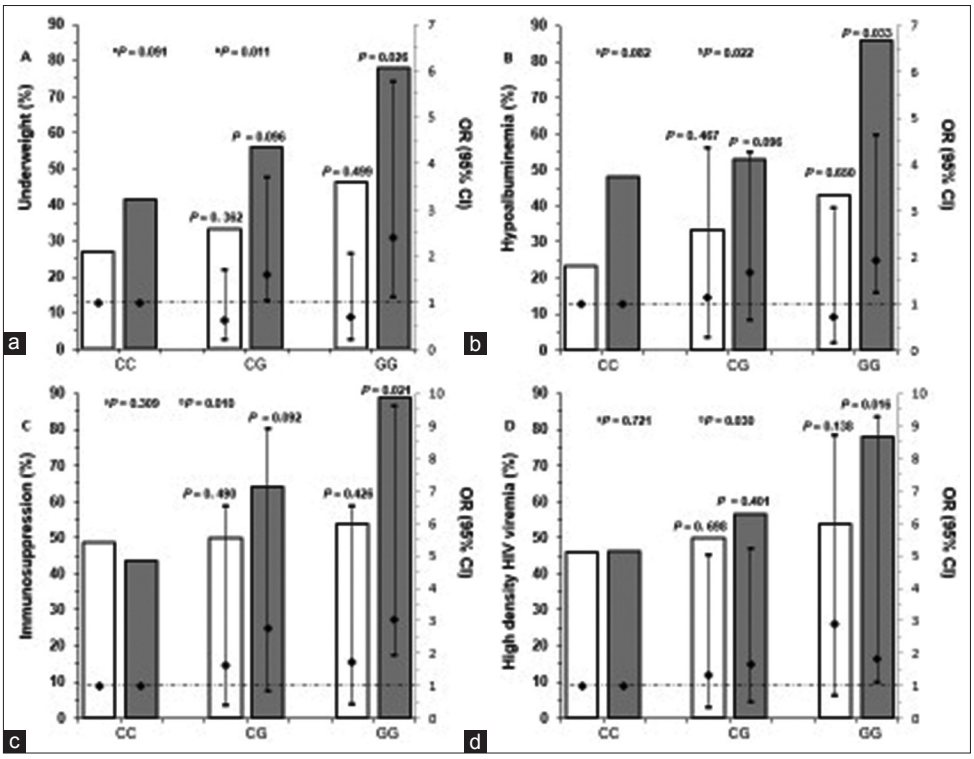

Figure 3: Frequency and association of underweight, hypoalbuminemia, immunosuppression, and high-density human immunodeficiency virus (HIV)-1 viremia with human ALB gene intron VII rs1445776009 genotypes with in antiretroviral treatment (ART)-experienced and -naive individuals. Data shown are combine bars and forest plot representing frequency of clinical syndromes and their association with the genotypes, respectively. Clear and gray bars represent ART naive and experienced with their respective odds ratio (OR) and 95% confidence interval (CI). [a]*P*, [b]*P*, and *P* are *P*-value of frequency clinical syndrome across the genotype in the ART naive and experienced and for association, respectively. (a) Underweight, (b) hypoalbuminemia, (c) immunosuppression, (d) high-density HIV-1 viremia. Heterozygous, CG, genotype and mutant, GG genotypes carriage. Binary regression analysis was conducted such that the clinical syndrome was entered as the dependent variables, CG or GG genotypes as the predictor variables, CC (wild-type) as the reference genotype controlling for age, gender, duration of substance injection, and duration of ART use. Bolded values indicate significant *P*-values

chronic ART use and substances use that depress the synthetic functions of the liver.[19] Reduction in serum albumin helps in transportation of drug such as ART to immunological tissues to reduce HIV-1 RNA multiplication and boost CD4+ T-cell function. Furthermore, proteolytic processing pro-albumin results EPI-X4 endogenous antagonist compound of CXCR4.[23] CXCR4 is very essential chemokine receptor, which plays integral role in immune system.[23] Further *in vitro* studies have shown substance use amplifies HIV-1 RNA copies replication.[48] Taken together, to high density HIV-1 viremia reduces CD4+ T cells.

Consistent with clinical markers, frequency of underweight, hypoalbuminemia, immunosuppression, and high-density HIV-1 RNA viremia was significantly high in the mutants, GG genotypes carriage relative to the wild-type CC, and genotype carriage in the experienced individuals. Further, analysis showed that the GG genotypes carriage associated with underweight, hypoalbuminemia, immunosuppression, and high-density HIV-1 RNA viremia relative to the wild-type CC, genotype carriage in the experienced individuals. The finding implies that mutant and GG genotype carriage at rs144577009 loci promotes development of HIV-1 infection in the ART-experienced ISUs. The possible reason could be variation at rs144577009 loci alters the binding site of transcription protein hence leading to gene deregulation. The consequences are likely more severe in the mutants and GG genotypes carriage. To the best of our knowledge, no single study has genotyped *ALB* gene in HIV-1 ISU. Therefore, the current study could be the first study to report on reduced serum albumin from genetic perspective in HIV-1-infected, ART-experienced, and -naive ISU. This study has few limitations, although the current study was cross-sectional, a prospective study design could be more effective since dietary intake could be monitored. Self-reported ISU and presence of scar could lead to bias. In addition, there was a need to carry out toxicological analysis of urine to quantify the concentration of both ART use and injection substance as well as strain of substances. Finally, recall memory in study participant, difficult to establish duration one has been living with HIV-1 infection prior testing.

Conclusion

This study found GG genotype carriage at loci ***rs1445776009*** of the *ALB* gene to be associated with serum albumin. These findings suggest that polymorphism in the intronic

variant ***rs1445776009*** could be modulating serum albumin levels and diseases outcomes in ART-experienced HIV individuals.

Authors' Declaration Statements

Ethics approval and consent to participate

Ethical approval of this study was taken from the ethical committee of Masinde Muliro University of Science and Technology, P. O. Box 190-50100, Kakamega, Kenya, and written informed consents were also taken from all involved participants.

Availability of data and material

The data used in this study are available and will be provided by the corresponding author on a reasonable request.

Authors' Contributions

TW and VB conceived and designed the study. NS and EB performed the experiments. EB performed statistical analyses, cointerpreted data, codrafted, and revised the manuscript with TW and NS. All authors have read and approved the manuscript.

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