(RESEARCH ARTICLE)

HIV and Malaria co-infection and the impact of viral load and HAART usage on the development of *Plasmodium falciparum* Artemisinin and Lumefantrine resistant genes in Nnewi, Anambra State, Nigeria

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Abstract

Background: Human Immunodeficiency virus (HIV) and malaria co-infection poses a serious health threat in sub-Saharan Africa and other endemic countries. Highly active anti-retroviral therapy (HAART) is currently used to suppress viral loads.

Methods: Blood samples collected from 400 participants comprising 200 HIV sero-positive and 200 sero-negative individuals was added to EDTA sample containers. Malaria parasitemia was evaluated using standard parasitological techniques followed by PCR techniques using the Quick Load One Taq One Step Polymerase Chain Reaction (PCR) for characterization of species of *Plasmodium* and resistant studies using specific primers. HIV viral load estimation was done using COBAS® TaqMan® Analyzer.

Results: Malaria has prevalent rate of 22.75% in the study population, while the prevalence of malaria infection among the HIV sero-positive and sero-negative is 77.0% and 23% respectively. Socio-demographic factors had no significant association with the development of resistant genes. HAART exposed individuals had prevalence of *Pf*K13 (6.9%) and *Pf*mdr-1 (20.8%). Viral load was significantly related with the development of resistant genes (100%) and (86.1%) for *Pf*K13 and *Pf*mdr-1 respectively.

Conclusion: Unsuppressed viral load in HIV sero-positive individuals heightens the prevalence of malaria parasitaemia and increases the chances of possible emergence and spread of *Pf*K13 and *Pf*mdr-1 genes.

Keywords: HIV; HAART; *Plasmodium falciparum*; Artemisinin; Lumefantrine

1. Introduction

HIV infection and malaria parasitaemia are among the commonest causes of global health challenges in the developing nations, which is estimated to have caused approximately 4 million deaths per year, with HIV increasing the burdensome and severity of malaria [1, 2, 3]. Malaria parasites are known to trigger the activation of CD4 cells and pro-inflammatory cells alongside signalling molecules such as cytokines, which in-turn promote favourable scenario for rapid HIV-1 replication in the CD4 cells[2].HIV positive pregnant women have great chances of developing symptomatic malaria, placental malaria and malaria transmission to children [4]. It is not clear if HIV infection can impact resistance

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to anti-malaria drugs such as Artemisinin, which is essentially employed for the treatment of malaria [5]. The recent achievements in the global reduction of the malaria burden are widely challenged by rising resistance to artemisinin and its derivatives [6-10].

Use of artemisinin-based combination therapy (ACT) such as hydroartemisinin-piperaquine has become a drug of choice for the treatment of malaria in the recent years [11]. It was on the premise of the success achieved with ACT that the National Malaria Control Programme declared that malaria would be completely eliminated by 2025, and the declaration has received consummate political support [12]. The emergence of resistant strains of *P. falciparum* to ACT poses grave threat to the success of this declaration [6]. Mutation of the K13 propeller gene encoding the F3D7/343700 kelch propeller (K13) domain has recently been implicated in artemisinin resistance [13, 14]. Delay in the clearance of parasites after 3 days of anti-malarial therapy is an indication of resistance [15]. There is also a possibility of partner drug resistance, which is the recurrence of parasitaemia in patients 4–6 weeks after initial malaria parasite clearance [16]. Resistant genes can also modify partner drugs due to polymorphic nature of certain genes e.g. single-nucleotide polymorphisms (SNPs) in *P. falciparum* chloroquine resistant transporter (*Pf*crt) and *P. falciparum* multi drug resistant-1 (*Pf*mdr1) for amodiaquine increase copy number of the *P. falciparum* multi drug resistant 2 (*Pf*mdr2) gene for Piperaquine, *Pf*mdr1 for Mefloquine [17-20]. It is also speculated that the abundance of multidrug resistant gene, *Pfmdr1* culminate with rise in Lumefantrine inhibition [17, 21, 22].

2. Methods

A population of 200 HIV-positive and 200 HIV-negative individuals all of which were 18months and above of age, attending clinics at Nnamdi Azikiwe University Teaching Hospital were recruited for the study. The ethical approval was obtained from the hospital's ethics committee with the approval number: NAUTH/CS/66/VOL.12/199/2019/055. The study population was divided into 2: Group A comprised of 200 HIV positive individuals on Highly Active Anti-Retroviral Treatment (HAART) and Group B comprised of 200 HIV negative individuals. Written consent was obtained from all the participants. Blood samples were collected, separated and processed following standard laboratory practices. Out of 4 mL of blood collected from the veins of participants, 2 mL were dispensed into a container of EDTA and mixed thoroughly. Bioline rapid diagnostic test (RDT) kit was used for malaria assay. Thick blood film was made for malaria parasite microscopy following WHO standard procedure. The viral load count for HIV positive subjects was done using the remaining part of the first 2ml volume of anti-coagulated blood previously collected for HIV sero-positive individuals. The second 2ml of blood was used for molecular identification of the malaria parasite, ACT resistant studies.

2.1. Preparation of Thick Blood Film

A drop of each of the well mixed blood samples were placed on the Centre of a clean grease free microscope glass slide (Malaria Diagnostic Workshop, 2010). A glass spreader was used to spread the blood in small circular form. The slide was kept to air dry. The blood films were stained with 3% Giemsa for 10mins, slides were then allowed to air dry.

Each of the overnight well dried thick film was placed back to back on the staining rack. Each slide was carefully flooded with three percent (3%) solution of the Giemsa stain made by adding 3ml of the stock solution to 97ml of water buffered to pH 7.2 and allowed to stain for 10mins. Clean water was gently poured on one end of the slide keeping off the film to avoid the film floating off the slide while washing off the stains. The washed slides were placed one by one with the film side facing downwards on a dry rack to dry and examined with x100 objective.

2.2. Detection of antibodies to HIV-1 and HIV-2

HIV serology was carried out using the current national algorithm for HIV sero-diagnosis. This involved the use of 3 rapid diagnostic kits, following their manufacturer's instructions. Briefly, each patient serum was screened for the presence of HIV antibodies using Determine® (Abott laboratories-kobe Japan). Serum which were negative to HIV antibodies, were considered negative for HIV. However, if it is positive for HIV antibodies, a second kit- Uni-gold (Trinity Biotech Plc, Bray, Ireland) was used. If the result of the second kit agrees with that of the first kit, the patient will be considered to be positive for HIV 1 and 2, but if it disagrees, Stat – Pak[R] (Chembio Diagnostic System USA) was used. The HIV sero-status of the patient was taken as a result of the third kit, either positive or negative.

2.3. Rapid Diagnostic Test (RDT)

P. falciparum antigen detection kit (Antech Diagnostics Limited, UK) was used for diagnosis according to the manufacturer's instruction. Two hundred microliters (200µl) of the sample, BioFluid and cell buffer (red) were added the micro tube alongside twenty microliters (20µl) of Proteinase K stock solution. The preparation was mixed thoroughly by voltexing and incubated at 55^0C for 10min. Equal volume of the genomic binding buffer was added to the

digested sample and vortexed for 10 seconds. The mixture was transferred to Zymo-Spin® II-xlr column in a collection tube, centrifugation was done at 12.000 x g for one minute, and the collection tube alongside flow through was discarded. Four hundred microliters (400µl) DNA Pre-wash buffer was added into the spin column of a new collection tube, centrifuged at 12,000x g for one minute. the collection tube was emptied. Seven hundred microliters (700µl) g-DNA wash buffer was added to the spin column and centrifuge at 12,000x g for one minute. This step was repeated with two hundred microliters (200µl) g-DNA wash buffer. The collection tube was discarded with the flow through. Spin column was transferred to a new micro-centrifuge tube with 50µl DNA Elution Buffer into the matrix and incubated for 5minutes at room temperature followed by centrifugation at maximum speed for one minute to elute the DNA

2.4. Polymerase Chain Reaction (PCR) Protocol for Plasmodium Species

Quick load One Taq one step PCR master (2X) with catalog number NEB MO486S was purchased from lnqaba Biotech Hartfield South Africa incorporated and used according to the manufacturer's instruction. Details of the primers used are stated below. The system components were thawed and mixed by inverting ten times. The PCR was performed in 50µl reaction mixture containing 25µl Quick Load One Taq one- step PCR master mix (2x), 1µL of each gene-specific forward primer (10µM), 1µL of each specific reverse primer10µM), 13ml of nuclease free water and 10µl of DNA template was added last. The PCR was started immediately as follows: Initial denaturation at 94^0C for 1minute, denaturation at 94^0C for 30secs, annealing at Tm-5 for 30secs, extension at 72^0C for 1minute, Go to the denaturation step for 39 cycles, final extension at 72^0C for 15mins and final holding at 4^0C.

Table 1 Primers used in detection of Plasmodium species

Primer Name	Primer Sequence
rMAL 1:	5'-ATA ACA TAG TTG TAC GTT AAG AAT AAC CGC-3'
rMAL 2:	5'-AAA ATT CCC ATG CAT AAA AAA TTA TAC AAA-3'
rOVA 1:	5'-ATC TCT TTT GCT ATT TTT TAG TAT TGG AGA-3'
rOVA 2:	5'-ATC TAA GAA TTT CAC CTC TGA CAT CTG-3'
rFAL 1:	5'-TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT-3'
rFAL 2:	5'-ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3'
rVIV 1:	5-CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC-3'
rVIV 2:	5' ACT CCA AGC CGA AGC AAA GAA AGT CCT TA-3'

Table 2 Resistance primers

Primer name	code	primary sequence	Expected yield
Pfmdr-1 F	S2270	TGCATCTATAAAACGATCAGACAAA	25
Pfmdr-1R	S2271	TCGTGTGTTCCATGTGACTGT	21
K13-F	S226E	GCCTTGTTGAAAGAAGCAGA	20
K13-R	S226R	GCCAAGCTGCCATTCATTTG	20

2.5. Electrophoresis of PCR products

One-point zero percent Agar-rose gel (1.0%) was prepared by dissolving 1.0g in 100ml Tris EDTA Buffer. The mixture was then heated in a microwave for 5minutes to dissolve completely. It was then allowed to cool at 56^0C and 6ul of Ethidium bromide was added to it. The Agar-rose gel was poured into the electrophoresis chambers with gel comb, and allowed to solidify.

Five micro liters of the amplified PCR products was analyzed on 1.5% Agar-rose gel containing Ethidium bromide in Tris EDTA buffer. Electrophoresis was performed at 90v for 60 minutes. After electrophoresis the PCR products were visualized by Wealth Dolphin Doc UV trans-illuminator and photographed. Molecular weights were calculated using molecular weight standard of the maker.

The PCR products were cleaned using Exonuclease/Shrimp Alkaline Phosphatase. Exonuclease/Shrimp Alkaline Phosphatase. Master mix was prepared by adding 50.0 μl Exonuclease I (NEB M0293) 20 U/ul and 200.0 μl Shrimp Alkaline Phosphatase (NEB M0371) 1U/ul in a 0.6 ml micro-centrifuge tube. Exonuclease/Shrimp Alkaline Phosphatase Mix of 2.5 μl was then added to 10.0 μl of PCR Mixture, mixed well and was incubated at 37°C for 30 minutes after which the reaction was stopped by heating at 95 °C for 5 minutes. Purification was done with the ABI V3.1 Big dye kit according to manufacturer's instructions. The labeled products were then cleaned with the ZymoSeq clean-up kit. Sequencing Binding Buffer of 240 μl was added to 20 μl sequencing reaction which was then transferred to a Zymo-Spin™ IB-96 plate mounted onto a collection plate. The mixture was centrifuged at 3,000 x g for 2 minutes. Sequencing Wash Buffer of 300 μl was added to each well of the plate and centrifuged at 3,000 x g for 5minutes. Fifteen microliter (15μl) of water was directly added to the column matrix of the filter plate. The ZymoSpin™ IB-96 plate was placed on top of the supplied 96-Well PCR plate and mount the assembly onto the Collection Plate which was then centrifuged at 3,000 x g for 2 minutes to elute the DNA.

2.6. HIV Viral load Estimation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 is based on three major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 permits automated specimen preparation followed by automated reverse transcription, PCR amplification and detection of HIV-1 target RNA and HIV-1 Quantitation Standard (QS) Armored RNA. The Master Mix reagent contains primers and probes specific for both HIV-1 RNA and HIV-1 QS RNA developed to ensure equivalent quantitation of group M subtypes of HIV-1 and of HIV-1 group O. The detection of amplified DNA is performed using target-specific and QS-specific dual-labeled oligonucleotide probes that permit independent identification of HIV-1 amplicon and HIV-1 QS amplicon. The quantization of HIV-1 viral RNA is performed using the HIV-1 QS. It compensates for effects of inhibition and controls the preparation and amplification processes, allowing a more accurate quantization of HIV-1 RNA in each specimen. The HIV-1 QS is a non-infectious Armored RNA construct that contains HIV sequences with identical primer binding sites as the HIV-1 target RNA and a unique probe binding region that allows HIV-1 QS amplicon to be distinguished from HIV-1 target amplicon. The HIV-1 QS is added to each specimen at a known copy number and is carried through the subsequent steps of specimen preparation, reverse transcription, simultaneous PCR amplification and detection of cleaved dual-labeled oligonucleotide detection probes. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer calculated the HIV-1 RNA concentration in the test specimens by comparing the HIV-1 signal to the HIV-1 QS signal for each specimen and control.

2.7. Data analysis

The data collected from the socio-demographic and clinical characteristics of the participants were entered into Microsoft Excel version 2016 and analyzed using STATA 16 statistical package 2019.

3. Results

Table 3 Prevalence of mono *Plasmodium spp* infection and mixed *Plasmodium spp* infection among the study population

Variable	Total	HIV positive (%)	HIV negative (%)	χ^2	p-value
Malaria infection					
Positive Negative	91(22.75) 309(77.25)	77(84.6) 123(40)	14(15.4) 186 (60.0)	56.460	<0.001*
Total	400	200	200		
Mixed/mono infection					
Pf only	51(12.75)	46 (90.2)	5 (9.8)		
Pvonly	1(0.25)	1 (100)	0(0)	58.113	<0.001*
Mixed Pf/Pv	39(9.75)	30 (77.0)	9 (23.0)		
Negative	309(77.25)	123 (39.8)	186 (60.2)		
Total	400	200	200		

The prevalence of mono and mixed malaria parasitaemia among HIV sero- positive and HIV sero- negative individuals is as shown on Table 1. Prevalence of *Plasmodium* spp in the study population is 22.75 % while that of *Plasmodium vivax* and *Plasmodium falciparum* are 0.25% and 12.75%respectively. The prevalence of *Plasmodium* mixed infection among the HIV sero-positive and sero-negative is 77.0% and 23% respectively. The HIV sero-positive had a significantly higher prevalence of *malaria* infection than HIV sero negative counterparts *(p<0.001)*.Plates 1 to 2 shows diversities of *Plasmodium species* among the study participants. *Plasmodium species* were analyzed through molecular genotyping. Two hundred samples were used for HIV sero-positive individuals and equally 200 samples from the HIV sero-negative individuals. The number of HIV sero-positive individuals with *Plasmodium falciparum* was 77 and 14 for HIV sero-negative individuals, (Plates 1 and 2). While *Plasmodium vivax* was 32 for HIV sero-positive individuals and 9 for HIV sero- negative individuals. (Tables 3 and 4) There was no infection with either *Plasmodium malariae* or *Plasmodium ovale*.

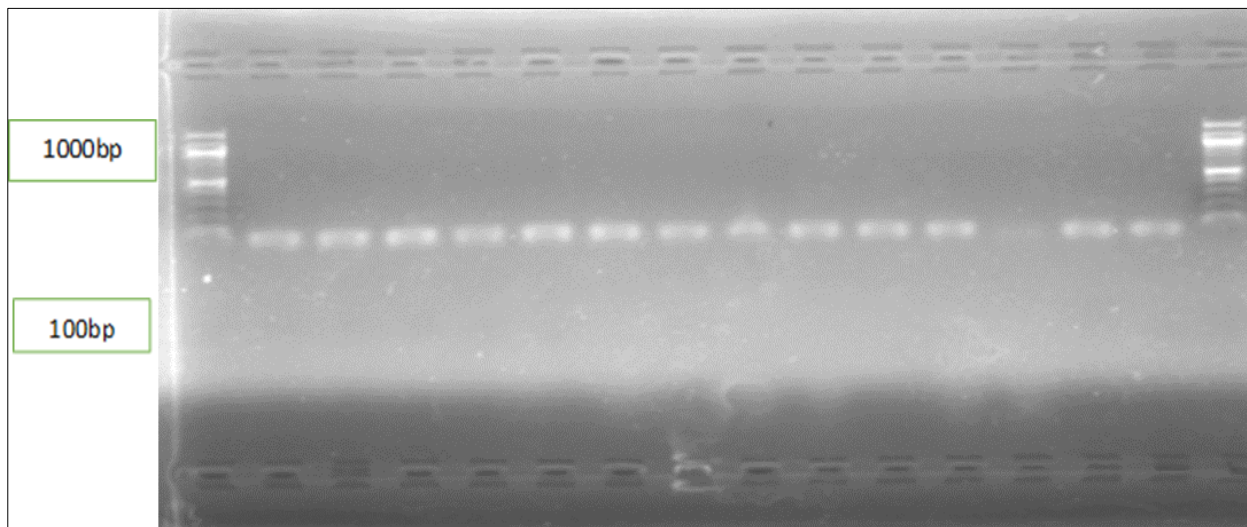**L 201 202 203 204 205 206 207 208 209 210 211 212 213 214 L**

Figure 1 Agar-rose gel bars of expressed *Plasmodium falciparum* genes analyzed on a 1.0 % agar-rose gel electrophoresis stained with ethidium bromide. L is a 100bp-1000bp DNA ladder (molecular marker). Samples 201, 202, 203, 204, 205,206, 207, 208, 209, 210, 211, 212, 213 and 214 are positive bands for the *expressed Plasmodium falciparum* genes at 100 bp. for HIV sero-positive individuals
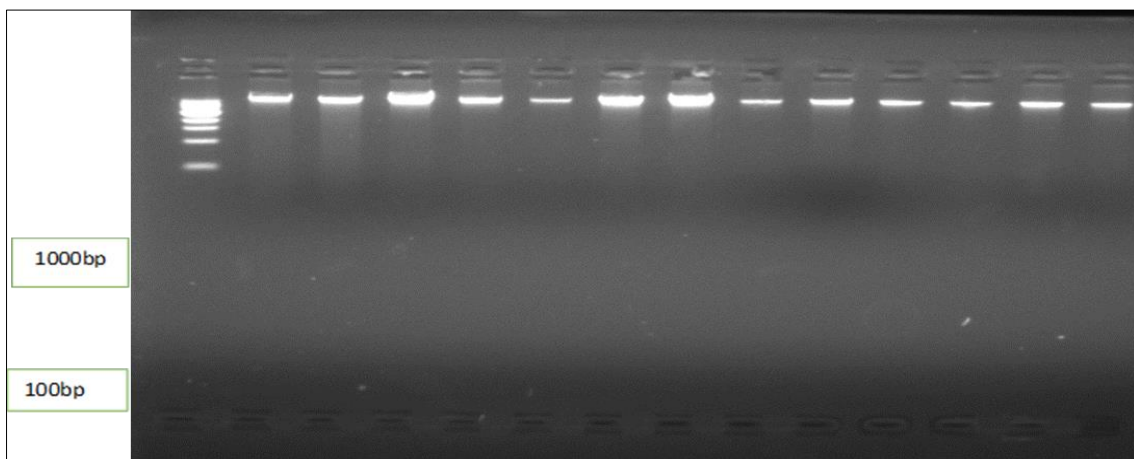
L 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure 2 Agar-rose gel bars of expressed *Plasmodium falciparum* genes analyzed on a 1.0 % agar-rose gel electrophoresis stained with ethidium bromide. L is a 100bp-1000bp DNA ladder (molecular marker). Samples 1, 2, 3, 4, 5, 6 7, 8, 9, 10, 11, 12, 13 and 14 are positive bands for the expressed *Plasmodium falciparum* genes at 100 bp for HIV sero- negative individuals
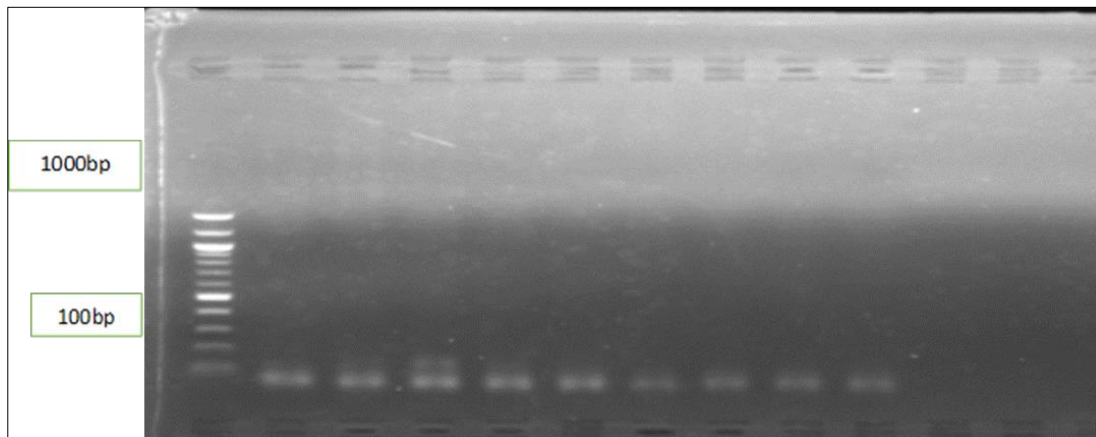
L 201 202 203 204 205 206 207 208 209

Figure 3 Agar-rose gel bars of expressed *Plasmodium vivax* genes analyzed on a 1.0 % agar-rose gel electrophoresis stained with ethidium bromide. L is a 100bp-1000bp DNA ladder (molecular marker). Samples 201, 202, 203, 204, 205,206, 207, 208, 209, are positive bands for the expressed *Plasmodium vivax* genes while 210, 211 and 212 are negative bands for the *Plasmodium vivax* genes at 100 bp for HIV sero- positive individuals
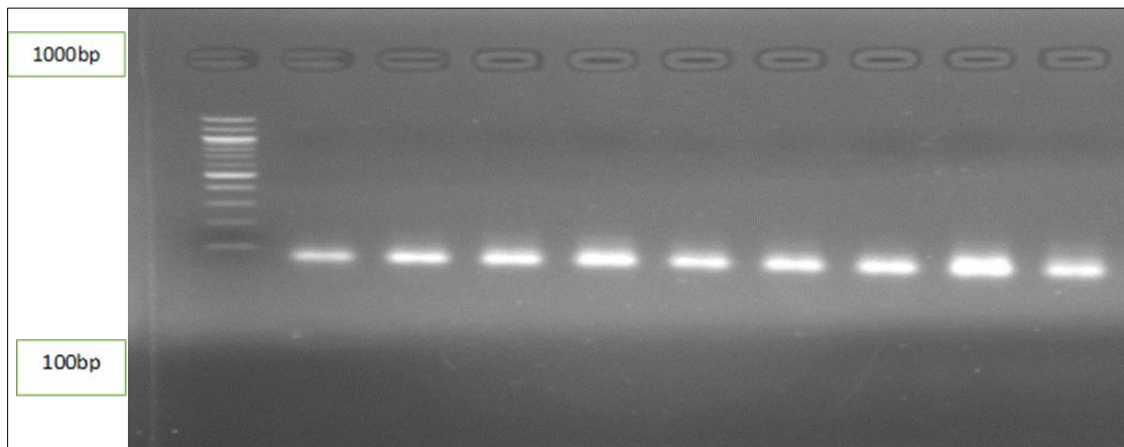
L 1 2 3 4 5 6 7 8 9

Figure 4 Agar-rose gel bars of expressed Plasmodium vivax genes analyzed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is a 100bp-1000bp DNA ladder (molecular marker). Samples 1, 2, 3, 4, 5, 6, 7, 8, and 9 are positive bands for the expressed Plasmodium vivax at 100 bp for HIV sero- negative individuals

Table 4 and 8 show the effect of HAART usage and duration of usage on the development of *Plasmodium falciparum* Artemisinin resistant gene (*Pf*k13), while tables 9 and 10 same effect on *Plasmodium falciparum* Lumefantrine (*Pf*mdr-1) genes among HIV-malaria co-infected individuals. 100 % of HIV positive individuals exposed to HAART had *Pf*K13 resistant gene while none was present in the HAART naïve group. Although there is no statistical relationship between HARRT usage and *Pf*K13 gene (*p*=0.376), there is an ox ratio value of 0.879,which indicate the likelihood that HARRT usage is a risk factor for the development of *Pf*K13 gene among HIV sero-positive individuals. There is also insignificant relationship between the time from onset of HARRT intake and the development of *Pf*K13 and*Pf*mdr-1 genes (*p*=0.293) and (*p*=0.547) respectively.

Table 8 shows the effect of immunity on the development of *Pf*k13 and *Pf*mdr-1 genes using viral load as immunological maker. All of the *Pf*K13 genes were recovered from HIV sero-positive individuals whose viral load were unsuppressed while that of *Pf*mdr-1 had 86.05% occurrence among HIV sero-positive individuals with unsuppressed viral load. There is a statistically significant relationship between the expression of *Pf*K13 gene and *Pf*mdr-1 gene and viral load at (p=<0.001)

Table 4 Effects of HAART usage on the development of *Pf*K-13 gene among HIV-Malaria co-infected individuals

*Pf*K-13	Total frequency (%)	HAART usage (%)		χ²	*p*-value
		Exposed	Naïve		
Positive	12 (6.0)	12 (100)	0(0)	1.992	0.376
Negative	188 (94.0)	161 (85.64)	27 (14.36)		
Total	200 (100)	173 (100)	27 (100)		

Statistically significant, p-value ≤ 0.05, OR=0.879(CI=0.102-3.285)

Table 5 Effects of HAART duration on the development of *Pf*K13gene among HIV-malaria co-infected individuals

*Pf*K-13	Duration of HAART (%)			χ²	p-value
	<10 years	10-20 years	21-30 years		
Positive	8 (66.7)	4 (33.3)	0	1.725	0.293
Negative	76 (47.2)	84 (52.0)	1 (0.62)		
Total	84	88	1		

Statistically significant, p-value ≤ 0.05 OR= 0.923(CI= 0.846- 3.568)

Table 6 Effects of HAART usage on the development of *Pf*mdr-1 gene among HIV-Malaria co-infected individuals

*Pf*mdr-1	HAART usage (%)		Total	χ²	p-value
	Exposed	Naïve			
Positive	36 (83.72)	7 (16.28)	43	0.362	0.547
Negative	137 (87.26)	20 (12.74)	157		
Total	173	27	200		

Statistically significant, p-value ≤ 0.05

Table 7 Effects of HAART duration on the development of *Pf*mdr-1 gene among HIV-malaria co-infected patients

*Pf*mdr-1	Duration of HAART (%)			Total freq (%)	χ²	p-value
	<10 years	10-20 years	21-30 years			
Positive	20 (55.6)	16 (44.4)	0	36	1.090	0.567
Negative	64 (46.7)	72 (52.6)	1 (0.70)	137		
Total	84	88	1	173		

Statistically significant, p-value ≤ 0.05

Table 8 Effect of immunity on the development of *Plasmodium falciparum* Artemisinin resistant gene (*Pf*K-13) and *Plasmodium falciparum* Lumefantrine resistant gene *Pf*mdr-1 using viral load immunological marker

Resistant gene	Viral load count		Total	χ²	p-value
	Suppressed (<1000)	Unsuppressed (≥1000)			
K-13					
Positive	0	12 (100.0)	12	52.39	<0.001*
Negative	161 (85.64)	27 (14.36)	188		
Total	161	39	200		

MDR-1					
Positive	6 (13.95)	37 (86.05)	157	153.70	<0.001*
Negative	155 (86.62)	2 (13.38)	43		
TOTAL	161	39	200		

Statistically significant, p-value ≤ 0.05

4. Discussion

Malaria and HIV co-infection has potentials of affecting the clinical outcome of each other. The impact of HIV on Malaria includes increase in severity, complications and decreased chances of patient's recovery, while malaria compromises immune system, enabling the rapid replications of HIV [23, 1]. Hence an understanding of how the two infections relate is needed for better and effective management and control of the two disease conditions. The emergence and spread of artemisinin-resistant *Plasmodium falciparum* from South East Asia (SEA) over a decade now poses a great threat to global malaria elimination and control [24]. The gains also made from the post HAART era in the management of HIV would be dampened should the Artemisinin resistant malaria become a public health issue among HIV sero-positive individuals.

The study population had more of females (69.5%) participants than male (30.5%) and HIV sero -positive (74.9%) and (25.1%) respectively. This is similar to the result by Simon-Oke *et al*, in a work carried out in Akure, Nigeria where they analyzed the impact of HIV associated immune-suppression on the *Plasmodium falciparum* chloroquine resistance transporter gene (*Pf*CRT) of HIV individuals and got28.08% males and 71.92% females [25]. More so, a good number of the study participants were observed to be traders 61.39% with secondary education 47.1% as the highest level of education. This could be as a result of the geographical region of the study group who are predominantly traders[26].

 HIV sero-positive individuals had 22.7% positivity for malaria parasites. This was lower than 38.5% and 60.6% in previous studies obtained in other studies in Nigeria [27, 28]. This variation can be explained by continued gradual decline of the prevalence of malaria as stated in a study by Dawaki *et al*, possibly due to the scale up of efforts towards prevention of malaria transmission in Nigeria over the years through the use of effective malaria prevention such as the use of insecticide treated mosquito nets and indoor residual spraying [27, 29].

The prevalence of malaria among HIV sero-positive individuals in this study was 84.6 %. This agrees with study done by Kwenti, where he studied Malaria and HIV co-infection in sub-Saharan Africa: prevalence, impact, and treatment strategies and found out that the prevalence of malaria among non-pregnant women was between 0.7%–47.5% overall [23]. Notwithstanding, the result is higher than 23.03%, 27.7%, 18.9%, and 7.8% recorded by Simon-Oke*et al*,Jegede *et al*, Onyenekwe *et al*, and Akinbo *et al*, respectively in Nigeria [25, 30-32]. It is equally higher; 74.3%, 47.7% and 54.2%, as recorded elsewhere in Nigeria by Dada and Srinivasan, Sanyaolu *et al*, and Omoti *et al*, respectively and 61.7% reported among HIV individuals infected with malaria in Mozambique [33-35].

The observed variation in sero prevalence of malaria among HIV individuals may be due to differences in geographical locations and time lapse between the studies. For instance the Nigerian studies conducted by Sanyaolu *et al*, Dada and Srinivasan, in South West and Omoti *et al*, in South –South, Nigeria had varying levels of prevalence [33, 34, 35]. However, the study by Onyenekwe*et al*, though carried out in same geographical region as this study shows a variation in prevalence most likely due to a long difference in year of the analysis and the non-usage of molecular technique which was employed in this study [29].

Molecular genotyping of *Plasmodium* indicated two *species*; *Plasmodium falciparum* and *Plasmodium vivax. Plasmodium ovale* and *Plasmodium malariae* species were not detected among the study participants. Among the entire study population, 12.75% had *Plasmodium falciparum* infection and only 1.0% had the *Plasmodium vivax* species. However, a mixed infection of *Plasmodium falciparum* and *Plasmodium vivax* of 9.75% was recorded. The discovery of *Plasmodium vivax* in the study not only bears evidence to the presence of *Plasmodium vivax in* Sub Sahara African countries, it should also provoke public health concern coupled with the need to retrain medical laboratory scientists who easily misdiagnose the infection since its presence was initially considered impossible in the Sub Sahara African countries [36, 37]. The findings of this study agrees with other works done in Cameroon by Howes *et al*, and Djeunang Dongho *et al*, and equally in the Southern Nigeria by Oboh *et al*, that shows incidence of *Plasmodium vivax* among Duffy coat negative population in Sub-Sahara Africa [47-50]. This is likely due to usage of alternative mechanisms to bind and invade erythrocytes or returning travelers from other regions [38-40]

By HAART exposure, 100% of the study participants who were exposed to HAART had *Plasmodium falciparum* artemisinin resistant gene (*Pf*K13) while none was present in the HAART naïve group. However, 83.72% prevalence rate was found for *Pf*mdr-1 among the study participants who were exposed to HAART. The length of time since the commencement of HAART usage also affected the development of the *Plasmodium falciparum* resistant genes with age group <10 years being the highest prevalence of 66.7% and 55.6% for *Pf*K13 and *Pf*mdr-1 respectively. There is however no significant relationship between the time from onset of HAART intake and the development of *Pf*K13 and *Pf*mdr-1 genes. This is similar to the study done by Simon-Oke *et al*, in Akure Nigeria were the impact of HIV-associated immune-suppression on *Plasmodium falciparum* chloroquine resistance transporter gene (*Pf*CRT) of HIV patients was analysed and 100% prevalence in patients with CD4count ≤ 200 was recorded [51]. While *Pf*mdr-1 had 86.1% prevalence rate from HIV sero-positive individuals with unsuppressed viral load. Viral load counts have remained one of the strongest correlates and surrogate marker of HIV disease progression regularly used in the clinical setting to monitor the infection. High viral load is a marker for rapid disease progression [41]. This agrees with the assertion of Shoko and Chikobvu, that Viral load and CD4 count can be used to ascertain helpful information needed to improve life expectancy of HIV patients [42].

5. Conclusion

There was a higher and significant association between malaria infection and HIV sero-positive individuals alongside a higher prevalence of mono or mixed infection of *Plasmodium falciparum* and *Plasmodium vivax.* However, it is noted that usage of HAART and duration of usage of HAART had no significant association on the development of artemisinin resistant gene *Pf*k13 and Lumefantrine resistant gene *Pf*mdr-1. The viral load of the HIV sero-positive individuals was significantly related to the development of resistant genes and as such this calls for serious concern among HIV sero-positive individuals with unsuppressed viral load.

Compliance with ethical standards

Author's contribution

Peace Amaka Onwuzurike is the prime investigator. Though the study was conceptualized by Professors Ifeoma Mercy Ekejindu and Ifeoma Bassie Enweani. Peace Amaka Onwuzurike generated the primary data. All authors helped in the validating and processing the raw data while Peace Amaka Onwuzurike prepared the manuscript.

Abbreviations

*Pf*mdr -1-*Plasmodium falciparum* Lumefantrine resistant gene
 HAART - Highly active anti-retro-viral therapy
*PF*K13 - *Plasmodium falciparum* Artemisinin resistant gene
L- Molecular ladder
HIV - Human Immunosuppressed Virus.
*Pf*CRT- *Plasmodium falciparum* chloroquine resistance transporter gene
NAUTH -Nnamdi Azikiwe University Teaching Hospital.

Disclosure of conflict of interest

All authors declare that they have no conflict of interest to disclose.

Statement of ethical approval

The study protocol was reviewed and approved by the Research Ethics committee of Nnamdi Azikiwe University Teaching Hospital Nnewi Anambra State Nigeria, with the following details: NAUTH/CS/66/VOL.12/199/2019/055.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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