Status of Anaemia and Malaria Co-infection With HIV From HAART Clinics in Federal Capital Territory, Nigeria: A Cross-Sectional Study

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Nneoma Confidence JeanStephanie Anyanwu[1,2], David Jesutobi Oluwatimileyin[1] and Peace Temitope Sunmonu[1]

[1]Department of Biological Sciences, Faculty of Science and Technology, Bingham University, Karu, Nigeria. [2]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

ABSTRACT

BACKGROUND: Malaria and HIV are 2 significant infections of critical public health concern globally. Malaria infection is one of the preceding causes of morbidity and mortality in endemic developing countries, and its co-infections in HIV patients worsen prognosis; with anaemia being the most common haematologic outcome of the infections.

CONTEXT AND PURPOSE OF STUDY: This study was aimed at determining the prevalence of anaemia and malaria co-infection among HIV-infected patients attending selected hospitals in Abuja between February and July 2019.

METHODS: A cross-sectional study was carried out to detect malaria in 420 HIV-positive patients who were 12 to 67 years old, using enzyme immunoassay and microscopy. A structured questionnaire was used to capture socio-demographic and risk factors ([Frequency of] Use of Malaria preventive Measures, History of anaemia, Blood type, malaria antecedents, and CD4+ Count) while packed cell volume was checked using micro haematocrit reader to determine anaemia status. Data were analysed using IBM SPSS v25.

RESULTS: The mean age of the study participants was 37.5 (±12.48). A total of 142 (33.8%) samples were positive for malaria, and 68 of the HIV-infected patients (16.2%) were anaemic; 4.8% of the 420 patients had malaria co-infection and anaemia simultaneously. More male participants had malaria co-infection (36.0%, $P = .617$) while more female participants had anaemia (22.7%, $P = .058$). Patients aged 61 to 70 years had the highest rates of malaria and those aged 51 to 60 years were most anaemic. Except for patients with normal CD4+ count, those who were more exposed to the evaluated risk factors were more co-infected and anaemic. Malaria co-infection did not significantly affect the onset of anaemia. Test for the validity of Microscopy against Enzyme Immunoassay (EIA) showed 83.1% sensitivity and 98.6% specificity. No association was observed between the variables and the parasitaemia density of the patients.

CONCLUSIONS: This study highlighted higher rates of malaria co-infection and anaemia among HIV patients when compared with previous reports in the region although co-infection did not significantly affect anaemia status. Given this trend, strategies must be put in place to checkmate these ailments. Population studies are also advocated.

KEYWORDS: Malaria, HIV, anaemia, co-infection, packed cell volume, *Plasmodium*, RDT, microscopy

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CORRESPONDING AUTHOR: Nneoma Confidence JeanStephanie Anyanwu, Department of Biological Sciences, Faculty of Science and Technology, Bingham University. PMB 005, Karu, Nasarawa State, Nigeria. Email: anyanwunneomaconfidence@gmail.com

Background

Malaria and HIV are 2 infections of global health concern. *Plasmodium falciparum* infection (malaria) is a life-threatening parasitic disease and more than 40% of the world population is living in malaria-prone areas.[1] It is considered a complex and overwhelming public health problem because it is one of the preceding causes of morbidity and mortality in endemic developing countries,[1] and its co-infections in HIV patients worsens prognostic outcome; with anaemia being the most common haematologic outcome of the infections. Anaemia is a deficiency in the number or quality of red blood cells and packed cell volume (PCV) is the percentage of red blood cells in the circulating blood. The co-infection of malaria and HIV could fast track the development of anaemia in the infected person. The HIV is easily transmitted through blood products and human to human interactions such as sexual intercourse. Anaemia is an important clinical problem in patients with HIV infection, caused by blood loss as a result of decreased RBC production, increased RBC destruction, and ineffective RBC production.[2]

Together, malaria and HIV cause more than 2 million deaths worldwide annually.[3] In malaria endemic areas, HIV infection increases the risk of co-infection with malaria in adults, especially in those with advanced immunosuppression.[4] Reports suggest that anti-malarial treatment failure may be more common among HIV patients with low CD4-cell counts and anaemic patients compared with those that are not infected with HIV.[3] Malaria is one of the leading causes of illness and death

in the world. Nine out of 10 of these deaths occur in Africa. It is the fourth leading cause of death of children under the age of 5 years and pregnant women in developing countries.[3] The HIV/AIDS is also one of the most destructive epidemics in the world, with about 5000 new infections per day.[4]

The infection of HIV is on the increase in sub-Saharan countries such as Nigeria. This region is also known to be endemic to malaria, particularly *P falciparum*. Considering this, it is not uncommon to observe co-morbidity with both pathogens. While infection with either malaria or HIV/AIDS can cause illness and death, infection with one can make infection with the other worse and/or more difficult to treat,[2] as malaria co-infection has been said to trigger disease progression in both infections, increase the chances of developing severe malaria in infected adults, fast track HIV replication both in vitro and in vivo, increase risk of congenital infection in pregnancy, as well as aid the spread of both diseases in endemic sub-Saharan African regions.[5,6] With a current population of 200 million and a declined HIV prevalence of 1.4%, malaria could pose a major threat to the progress made on HIV prevention and management in Nigeria over the years. Very few Nigerian studies[6-13] have reported malaria co-infection with HIV, most of which were studies done in the southern part of the country. No study has reported the status of malaria co-infection with HIV in Abuja, to the best of our knowledge. Anaemia in HIV-infected patients can have serious implications, which vary from functional and quality-of-life decrements to an association with disease progression and decreased life span. This study was therefore aimed at determining the prevalence of malaria co-infection among HIV-infected patients attending selected hospitals in Abuja, as well as its effect on their anaemia status.

Materials and Method

Study location, design, and population

This study was carried out among HIV-infected patients attending 3 selected hospitals in the Federal Capital Territory (FCT) Abuja, Nigeria. Federal Capital Territory is the capital of Nigeria, formed in 1991 from parts of its neighbouring states, namely, Nasarawa, Niger, and Kogi. Flanked by less-developed and population-dense suburbs, malaria transmission rate in Abuja is irregular. Malaria epidemics can occur when climate and other conditions suddenly favour transmission in areas where people have little or no immunity to malaria or when susceptible individuals move into areas with intense malaria transmission like slums with conducive habitats for female anopheles mosquitoes. The hospitals selected for this study were Garki Hospital, Abuja, Maitama District hospital, and Gwarinpa General Hospital. The hospitals are referral centres for the management of HIV-positive patients, hence their selection. A cross-sectional study was carried out on HIV-positive patients using random sampling technique between February and July 2019. The patients were aged 12 to 67 years old and were registered in the HAART (highly active antiretroviral therapy) clinics of the selected hospitals. Their HAART regimens comprised of Lamivudine, Zidovudine, and Nevirapine.

Ethical approval and informed consent

Written informed consent was obtained from the participating adult patients and the guardians of the minors (patients <18 years); and ethical clearance was obtained from the FCT Health Research Ethics Committee with the code FHREC/2019/01/22/23-03-2019. Additional approvals were also obtained from the local ethics committees of the selected medical centres. The clearance codes are as follows: FCTA/HHSS/GGH/GEN/05 (Gwarinpa General Hospital), FCTA/HHSS/HMB/GEN/038/T (Maitama District Hospital), and GHA/HR/GEN/0074/19 (Garki Hospital Abuja).

Inclusion and exclusion criteria

Inclusion criteria. Patients who were HIV-positive undergoing HAART in the selected hospitals at the time of the study were included. Patients who exhibited malaria symptoms but who had not undertaken malaria testing were included. All the patients included were aged 12 to 67 years and gave their consent. Solely underage patients whose guardians/caregivers consented to their participation in the study and who conceded to voluntarily participate were included in the study.

Exclusion criteria. Patients who did not give their consent, those above the maximum age, HIV-negative individuals, and people who indicated interest after the maximum sample size was obtained. Patients with AIDS-defining conditions were excluded. Patients who had very recent positive (untreated) test to malaria were excluded to avoid bias. All minors whose parents refused to allow them to partake in the study and/or those who refused to participate in the study were also excluded.

Sample collection and processing

Samples were randomly collected from anonymous HIV-positive individuals who had an axillary temperature of ⩾37°C, along with their socio-demographic data and responses to risk factors via structured questionnaires. The CD4+ T-cell counts of the patients were obtained from their medical records; 5 mL of venous blood from the HIV-infected patients was collected into K2 EDTA vacutainer tubes via venipuncture by the staff of the various hospitals and transferred immediately to the Department of Biological Sciences, Bingham University, Karu, for further processing. These samples were collected from 420 infected patients, confirmed to be HIV seropositive, and screened for the presence of IgM, IgG, and IgA antibodies to malaria using an enzyme immunoassay cassettes (OnSite Malaria Pf/Pv Ab Combo Rapid Test, CTK Biotech, CA 92121, USA) based on double antigen lateral flow chromatographic immunoassay principle. Two red lines on the test (T) and control (C) regions indicated a positive

result, while a single line on the C region indicated a negative result. The whole blood samples were also confirmed via microscopy for comparative purposes using Leishman stain.[14] The PCV of the patients were then determined using the microhaematocrit method[12] to determine anaemia status.

Rapid tests

All samples were confirmed seropositive to HIV using Uni-Gold Recombigen HIV 1/2 (Trinity Biotech Plc, Bray, Ireland), Determine (Alere Medical Company Ltd, Japan), and then Multispot HIV 1/2 (Bio-Rad Laboratories, Redmond, WA) rapid tests.

For malaria detection, the enzyme immunoassay cassette was labelled with sample codes, a capillary tube was used to vertically draw whole blood specimen and the blood was placed on the sample well of the cassette. Two drops of sample diluent were immediately added to the sample well. The results were read within a minimum of 15 minutes and a maximum of 30 minutes (per manufacturer's instructions) and documented afterwards. The cassette contains a built-in control feature, the C line. The C line developed after addition of specimen and diluent. The test was duplicated with fresh cassette. The kit used was for IgG, IgM, and IgA detection of *P falciparum* and *P vivax*.

Microscopy

Thin blood films were made on sterile slides and stained with Leishman stain; fixed with absolute anhydrous methanol and left to sit for 2 minutes, rinsed with distilled water, then left to air dry. Slides were viewed under the light microscope at a magnification of ×100 using oil immersion. A definitive parasitaemia was reported as the presence of nuclear chromatin pattern (red chromatin dot with purple cytoplasm of the parasite) of cells. The percentage malaria parasite (MP) density was estimated against RBC by counting the parasitized cells against 1000 RBCs. Twenty fields were screened and percentage parasitaemia was expressed as

$$\frac{parasitized\ \ RBCs}{total\ \ RBCs}\times 100$$

Density was grouped as high (>10 parasites/1 field), moderate (1-10 parasites/1 field) and low (1-10 parasites/10 fields),[15] and expressed in means. The CD4+ T-cell counts of the patients were obtained from their medical records.

Determination of the sensitivity and specificity between microscopy and enzyme immunoassay rapid test kit

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the microscopy and rapid immunochromatographic assays from the present study were determined using the formulae below

$$\text{Sensitivity or True Positive Rate } (\text{TPR}) = \frac{\text{TP}}{\text{TP}+\text{FN}}\times 100$$

$$\text{Specificity or True Negative Rate } (\text{TNR}) = \frac{\text{TN}}{\text{TN}+\text{FP}}\times 100$$

$$\text{Positive predictive value } (\text{PPV}) = \frac{\text{TP}}{\text{TP}+\text{FP}}\times 100$$

$$\text{Negative predictive value } (\text{NPV}) = \frac{\text{TN}}{\text{TN}+\text{FN}}\times 100$$

$$\text{Accuracy} = \frac{\text{TP}+\text{TN}}{\text{TP}+\text{FP}+\text{FN}+\text{TN}}\times 100$$

where TP is True Positive; TN is True Negative; FP is False Positive; and FN is False Negative.

Packed cell volume

The patients' recent history of anaemia was obtained from their medical records. This was done to eliminate anaemia of microcytic, macrocytic, and normocytic (abnormal morphology) origin, as well as those resulting from unrelated diseases and polydipsia. Blood was collected into heparinized microhaematocrit tube; two-third of the tube was filled, one end of the tube was sealed with a clay sealant, placed into a calibrated micro haematocrit centrifuge and centrifuged for 3 to 5 minutes at 10 000 r/min. Haemolysis was noted, as this may lower the haematocrit results regarding the haemoglobin (the haematocrit is 3 times the value of the haemoglobin if the cells are normocytic). A lined card, the PCV reader, was used to determine the haematocrit value. They all work by the same principle; the height of the total blood column and the height of the red cell layer were measured. A PCV < 33% was reported to be anaemic while a PCV of < 15% was reported as severe anaemia.[16]

Statistical analysis

A preliminary analysis of the data was done and presented in integers (whole numbers) and proportions. Prevalence of malaria and anaemia in the study population were summarized with the use of percentages. All percentages were calculated within groups of variables. The definite parameters between anaemic patients and patients co-infected with malaria were compared using Pearson chi-square test. Logistic regression analysis was used to examine the relationship between anaemia, malaria co-infection, and the risk factors. All data were analysed using IBM SPSS version 25 (SPSS Incorporated, Chicago, IL, USA). Generated data were presented in tables. The *P* values were considered statistically significant at <.05 (95% confidence interval).

Results

Among the 420 HIV patients examined, 150 (35.7%) were male and 270 (64.3%) were female patients. The target range for this study was patients aged 12 to 67 years and 142 (33.8%) were positive for *P falciparum* employing microscopy while 122

Table 1. Distribution of packed cell volume among HIV-malaria co-infected and anaemic patients in the selected hospitals.

PERCENTAGE PCV	NUMBER EXAMINED	NUMBER CO-INFECTED (%)	NUMBER ANAEMIC, PCV < 33 (%)	NUMBER CO-INFECTED + ANAEMIC, PCV < 33 (%)
10-19	2	0 (0.0)	2 (100.0)	0 (0.0)
20-29	22	12 (54.5)	22 (100.0)	12 (54.5)
30-39	272	88 (32.4)	44 (16.2)	8 (2.9)
40-49	124	42 (33.9)	0 (0.0)	0 (0.0)
Total	420	142 (33.8)	68 (16.2)	20 (4.8)

Co-infection: $\chi^2 = 2.753$, $df = 3$, $P = .431$; Anaemia: $\chi^2 = 71.884$, $df = 3$, $P = .000$; Co-infection + Anaemia: $\chi^2 = 0.351$, $df = 3$, $P = .554$. Abbreviation: PCV, packed cell volume.

(29%) had antibodies to the parasite, as observed using enzyme immunoassay cassette rapid test kit. Among the 420 patients sampled, there was no *P vivax* seen via microscopy and rapid diagnostic testing (RDT), hence *P vivax* had zero prevalence in the population studied. The overall rate of anaemia in the population studied was 16.2% (68 of 420) while the overall anaemia + malaria co-infection rate was 4.8% (20 of 420) (Table 1).

Patients aged 61 to 70 years had the highest rate of parasitaemia (46.7%), while patients aged 51 to 60 years had the highest rate of anaemia (19.0%). The patients aged 21 to 30 years old had the least co-infection rate (26.8%), while patients <20 years old had the least percentage frequency of anaemia (Table 2). The prevalence rates of malaria and anaemia were not associated with the age of the patients ($P > .05$).

More male patients (36.0%) than female patients (32.6%) had malaria co-infection while a higher rate of anaemia was seen among female patients ($P > .05$) (Table 2). The distribution of malaria co-infection and anaemia showed that the 2 uneducated patients captured in this study were anaemic and co-infected with malaria. No co-infection cases were found among patients who had informal education, although 2 (50.0%) of them had anaemia. The rest of the patients with different educational qualification had varying rates of malaria co-infection and anaemia (Table 2). The frequency of co-infection and anaemia among the patients were not significantly associated with the patients' level of education ($P > .05$). Although no statistical significance was observed, patients resident in urban areas had slightly higher malaria co-infection (34.4%) than rural dwellers (33.3%), and anaemia was preponderant among rural settlers (17.9%) than urban dwellers (14.0%) ($P > .05$) (Table 2).

Logistic regression analysis revealed that the likelihood of developing co-infection was higher in men than women (odds ratio [OR] = 1.094), and patients aged 61 to 70 years were most likely to have malaria co-infection (OR = 1.125). Patients who did not take preventive measures were more co-infected with malaria (41.4%) than those who did (30.0%). However, the likelihood of developing co-infection was low (OR = 0.446). Patients who used preventive measures ⩾ 1 month intervals had the highest frequency of malaria co-infection (42.4%), and the likelihood of occurrence was high (OR = 1.340). Those who had history of anaemia, SS blood type, malaria antecedents, and normal CD4+ count had higher rates of anaemia and malaria co-infection. The likelihood of having malaria co-infection was significantly high among patients who had previous history of anaemia (OR = 11.455, $P = .039$). Patients who had normal CD4+ count were more co-infected with malaria than the other groups (38.9%). They also had the highest rate of anaemia (18.1%). However, there was no association between CD4+ count and the occurrence of malaria co-infection and anaemia from this study (Table 3). Patients who were more predisposed to malaria-related risk factors had higher frequency of anaemia than those who were less exposed to risk factors. They were less likely to have anaemia based on their exposure to these factors (OR < 1) (Table 3).

The test for the validity of microscopy and rapid immunochromatography assay revealed sensitivity and specificity to be 83.1% and 98.6%. Twenty-four patients who were negative for malaria infection via rapid test were found to be positive using microscopy while 4 patients who were found to be positive via rapid immunochromatography assay were confirmed negative using microscopy. The PPV and NPV were 96.7% and 91.9%, respectively (Table 4). None of the patients had a high MP density, and the ratio of low-density parasitaemia to moderate density parasitaemia was 6.89:1 (Table 5). No association was observed between the variables and the MP density of the patients.

Discussion

The prevalence of malaria in patients attending 3 major hospitals in Abuja, FCT, was observed to be 33.8% (142 of 420). The co-infection rate from this study is extremely high (33.8%), compared with the 2.9% obtained in Lagos[6]; the 7.8% reported in Kogi[10]; the 2.11% and 9.78% found in Benin City, Nigeria[8,9]; the 18.81% reported by Chinedum et al[13]; and the 4% observed in Uganda.[17] A higher co-infection rate (43.96%) than that obtained from this study has however been found in South-Eastern Nigeria by Ukibe et al.[12] The high rate of malaria co-infection seen in this study could be indicative of the increased exposure of HIV-infected patients to the parasite. The immunosuppression

Table 2. Distribution of malaria co-infection and anaemia according to the HIV/AIDS patients' socio-demographic features.

VARIABLE	NUMBER EXAMINED	MALARIA CO-INFECTION (%)			ANAEMIA (%)		
		NUMBER POSITIVE (%)	χ^2	*P* VALUE	NUMBER POSITIVE (%)	χ^2	*P* VALUE
Age group (in years)							
⩽20	22	10 (45.5)	4.060	.541	2 (9.1)	1.025	.961
21-30	112	30 (26.8)			16 (14.3)		
31-40	140	48 (34.3)			24 (17.1)		
41-50	74	22 (29.7)			14 (18.9)		
51-60	42	18 (42.9)			8 (19.0)		
61-70	30	14 (46.7)			4 (13.3)		
Total	420	142 (33.8)			68 (16.2)		
Sex							
Male	150	54 (36.0)	0.250	.617	34 (12.6)	3.606	.058
Female	270	88 (32.6)			34 (22.7)		
Total	420	142 (33.8)			68 (16.2)		
Place of living							
Urban	186	64(34.4)	0.027	.870	26 (14.0)	0.602	.438
Rural	234	78 (33.3)			42 (17.9)		
Total	420	142 (33.8)			68 (16.2)		
Educational qualification							
None	2	2 (100.0)	3.649	.456	2 (100.0)	8.354	.079
Primary	124	42 (33.9)			18 (14.5)		
Secondary	144	44 (30.6)			28 (19.4)		
Tertiary	146	54 (37.0)			18 (12.3)		
Informal	4	0 (0.0)			2 (50.0)		
Total	420	142 (33.8)			68 (16.2)		

accustomed to HIV infection increases the risk of co-infection with other pathogens, and *P falciparum* infection is endemic in the region; hence, co-infection in HIV-positive patients is not far-fetched. It would be expected that HAART regimen would boost the immunity of these patients and shield them from further infections, especially with the development of regimen such as Lamivudine and Nevirapine which possess an inhibitory effect on *Plasmodium*,[8,10] as well as Co-trimoxazole prophylaxis. The possibility of these patients exposing themselves to predisposing factors should however not be overlooked.

The co-infection pattern according to age was highest among 61 to 70 age group. Their waning immunity, coupled with possible increased exposure to risk factors of malaria may be responsible for this finding, because, irrespective of immune status, the infection cannot occur without prior exposure to predisposing factors.

Analysis of sex-related prevalence of malaria among the HIV-infected patients in this study revealed that more men than women were co-infected. This finding corresponds with the findings of Akinbo et al[9] and Wariso,[18] but contradicts the report of Saracino et al[19] who found higher co-infection rate in women. The finding from this study is most likely due to men having more exposure to the parasite as a result of day-to-day activities which could extend till late in the night. The average man has greater amount of night life than the women in the metropolitan city of Abuja. The vectors of these parasites also thrive better at night as they are nocturnal, thus, bites/infection occur more at night than during the day, making men easier targets. Although more of the female patients reported to the hospital for HAART than the male patients, the higher co-infection in male patients may be due to the higher frequency of exposure to risk factors associated with blood type, malaria

Table 3. Logistic regression relationship between anaemia, malaria, and selected factors among HIV-infected patients.

FEATURES	GROUP/RESPONSE	NUMBER EXAMINED	MALARIA CO-INFECTION				ANAEMIA			
			NUMBER POSITIVE (%)	OR	95% CI	*P* VALUE	NUMBER POSITIVE (%)	OR	95% CI	*P* VALUE
Age group (in years)	⩽20	22	10 (45.5)	1.125	0.896-1.411	.311	2 (9.1)	0.928	0.679-1.268	.639
	21-30	112	30 (26.8)				16 (14.3)			
	31-40	140	48 (34.3)				24 (17.1)			
	41-50	74	22 (29.7)				14 (18.9)			
	51-60	42	18 (42.9)				8 (19.0)			
	61-70	30	14 (46.7)				4 (13.3)			
Sex	Male	150	54 (36.0)	1.094	0.586-2.041	.778	34 (12.6)	0.571	0.251-1.299	.182
	Female	270	88 (32.6)				34 (22.7)			
Educational qualification	None	2	2 (100.0)	0.966	0.675-1.383	.851	2 (100.0)	1.223	0.744-2.010	.428
	Primary	124	42 (33.9)				18 (14.5)			
	Secondary	144	44 (30.6)				28 (19.4)			
	Tertiary	146	54 (37.0)				18 (12.3)			
	Informal	4	0 (0.0)				2 (50.0)			
Use of malaria preventive measures	Yes	280	84 (30.0)	0.446	0.167	.107	44 (15.7)	0.683	0.177	.580
	No	140	58 (41.4)		1.191		24 (17.1)		2.638	
Frequency of use of preventive measures	None	140	58 (41.4)	1.340	0.871	.183	24 (17.1)	0.990	0.549	.973
	Daily	98	20 (20.4)		2.062		16 (16.3)		1.783	
	Weekly-Fortnightly	116	36 (31.0)				24 (20.7)			
	⩾Monthly	66	28 (42.4)				4 (6.1)			

(Continued)

Table 3. (Continued)

FEATURES	GROUP/RESPONSE	NUMBER EXAMINED	MALARIA CO-INFECTION				ANAEMIA			
			NUMBER POSITIVE (%)	OR	95% CI	*P* VALUE	NUMBER POSITIVE (%)	OR	95% CI	*P* VALUE
History of anaemia	Yes	12	10 (83.3)	11.455	1.126	.039*	12 (100.0)	0.000	0.000	.999
	No	408	132 (32.4)		116.518		56 (13.7)		.	
Blood type (Genotype)	AA	168	58 (34.5)	0.874	0.487	.653	24 (14.3)	0.761	0.332	.519
	AS	248	82 (33.1)		1.570		40 (16.1)		1.745	
	SS	4	2 (50.0)				4 (100.0)			
Frequency of malaria in a year	None	6	0 (0.0)	0.956	0.720	.756	0 (0.0)	0.891	0.619	.533
	Once	184	66 (35.9)		1.269		30 (16.3)		1.282	
	Twice	114	38 (33.3)				16 (14.0)			
	Thrice	70	26 (37.1)				14 (20.0)			
	>Thrice	46	12 (26.1)				8 (17.4)			
CD4+ count	<200	78	16 (20.5)	1.197	0.673	.541	10 (12.8)	0.923	0.568	.487
	200-499	126	42 (33.3)		2.127		19 (15.1)		2.201	
	>500	216	84 (38.9)				39 (18.1)			

Abbreviations: CI, confidence interval at 95%; OR, odds ratio.
*Significant.

preventive measures, and social behaviour.[10] There was however no statistically significant association ($P > .05$) between sex and malaria co-infection. The finding may also be due to the epidemiological differences in the different study population and variation in methodology.

There was a slightly higher co-infection rate among the urban dwellers from this study. The reason for our findings could be the fact that malaria prevention strategies undertaken by international organizations and nongovernmental organizations (NGOs) in Nigeria are more concentrated in the volatile rural areas; thus, the outcome could have improved over the years. Furthermore, people living in urban areas key into the popular misconception that urban areas are less prone to infectious diseases. Most of those living in urban areas see the use of long-lasting insecticide-treated mosquito nets (LLIN) as archaic and demeaning to the aesthetics of their rooms.

Except for patients with normal CD4+ count, patients who were more predisposed to risk factors associated with malaria infection (ie, patients who did not use preventive measures, those who employed preventive measures monthly, who had history of anaemia, had SS blood types, and had malaria antecedents) were more co-infected with parasitaemia and had higher frequency of anaemia than those who were less exposed to risk factors from the logistic regression model. Except for their educational qualification, the likelihood of having anaemia based on their exposure to the evaluated risk factors was low (OR < 1). Non-adherence to strict preventive measures (ie, daily use of LLIN, mosquito repellent, and/or insecticides) increases the chance of malaria infection. Exposure to risk factors also increases the chance of co-infection. The highest co-infection rate found among patients with SS blood type might be due to frequent exposure to the parasite's vector. Sickle cell

Table 4. Measure of validity of microscopy and RDT assays used.

CASSETTE	MICROSCOPY		
	TEST NEGATIVE	TEST POSITIVE	TOTALS
Test positive	4	118	122
Test negative	274	24	298
Totals	278	142	420

Sensitivity = 83.1%, Specificity = 98.6%, PPV = 96.7, NPV = 91.9. Abbreviations: NPV, negative predictive value; PPV, positive predictive value; RDT, rapid diagnostic testing.

Table 5. Malaria parasite density among co-infected HAART patients.

FEATURES	RESPONSE	NUMBER EXAMINED	NUMBER POSITIVE (%)	MP DENSITY (%)			χ^2	*P* VALUE
				LOW (0-999 μ/L)	MODERATE (1000-9999 μ/L)	HIGH (>10 000 μ/L)		
Sex	Male	150	54	48 (32.0)	6 (4.0)	0 (0.0)	2.616	.106
	Female	270	88	76 (28.1)	12 (4.4)	0 (0.0)		
Age group	⩽20	22	10	8 (36.4)	2 (9.1)	0 (0.0)	0.296	.961
	21-30	112	30	26 (23.2)	4 (3.6)	0 (0.0)		
	31-40	140	48	42 (30.0)	6 (4.3)	0 (0.0)		
	41-50	74	22	19 (25.7)	3 (4.1)	0 (0.0)		
	51-60	42	18	16 (38.1)	2 (4.8)	0 (0.0)		
	61-70	30	14	13 (43.3)	1 (3.3)	0 (0.0)		
Blood type	AA	168	58	46 (27.4)	12 (7.1)	0 (0.0)	0.729	.694
	AS	248	82	76 (30.6)	6 (2.4)	0 (0.0)		
	SS	4	2	2 (50.0)	0 (0.0)	0 (0.0)		
CD4+ count	<200	78	16	16 (20.5)	0 (0.0)	0 (0.0)	1.366	.713
	200-499	126	42	36 (28.6)	6 (4.8)	0 (0.0)		
	>500	216	84	72 (33.3)	12 (5.6)	0 (0.0)		
Anaemia status	Anaemic	68	20	10 (14.7)	10 (14.7)	0 (0.0)	2.787	.426
	Non-anaemic	352	122	110 (31.3)	12 (3.4)	0 (0.0)		

Abbreviations: HAART, highly active antiretroviral therapy; MP, malaria parasite.

trait (AS genotype) is known to confer protection against malaria infection to carrier,[20] hence, the least frequency of co-infection obtained within this group was not surprising. Having a previous history of anaemia was a predictor of HIV-malaria co-infection from this study (11.455, $P = .039$), indicating that patients who had previous history were more prone to co-infection. This contradicts previous findings that anaemia confers protection against malaria infection.[21,22]

The higher rate of co-infection observed among non-anaemic patients (34.7%) compared with the anaemic patients (29.4%) depicts that being infected with malaria does not negatively affect the anaemia status of HIV-infected patients. As our study did not include HIV seronegative individuals, we were unable to ascertain the relationship between malaria and anaemia in the absence of HIV co-infection. However, we deduced from the increased prevalence of anaemia in those who had a history of anaemia, as well as the 2 severe cases of anaemia in patients not co-infected with malaria that HIV contributes to making patients anaemic. Various factors including type of HAART regimen (eg, Zidovudine [ZDV]), antibody production to HAART agents contribute to anaemia in HIV-positive patients,[10] as well as CD4+ T-cell count (<200 cells/μL); white blood cell (WBC) and platelets count of less than 4000 and 200 000 cells/μL, respectively; and duration of HAART. Reports show that the prevalence of anaemia among adult HIV/AIDS patients lies between 23% and 50% worldwide, and 24% and 58% in Africa. Similar to the overall frequency of anaemia obtained from our study, an Ethiopian study has shown the prevalence of anaemia to be 16.2% among HIV/AIDS patients[23] while an overall anaemia prevalence of 60.61% was observed in Benin City, Nigeria.[24] The higher occurrence of anaemia in women in this study may be due to the higher frequency of exposure to risk factors associated with the disease progression including pregnancies, immune status, body mass index (BMI) levels, and genotype. The preponderance of anaemia among the rural dwellers could be attributed to their standard of living or economic status, which could aggravate the severity of their co-infection, leading to increased frequency of anaemia.

The sensitivity and specificity obtained from this study is an eye-opener for Abuja and its neighbouring states, as the routine diagnosis of *P falciparum* malaria is predominantly based on RDT in the region. The alarming rate of false-negative RDT (FN-RDT) results could be due to low parasite density infection and deletion of the pfhrp2/3 gene.[25] Low transmission intensity and high urbanicity could also have contributed to the FN-RDT results observed, as Abuja is a metropolitan city. Microscopy is the gold-standard for malaria diagnosis[26]; hence, the higher sensitivity and specificity obtained in this study is not surprising.

No association was observed between the variables and the MP density of the patients. In non-immune/immunocompromised patients, symptomatic malaria can ensue at low parasite densities, and high parasitaemia can take a serious turn in anaemia. The relative lack of association between the density of malaria and anaemia in this study could be attributed to the absence of high-density malaria and the low rate of moderate-density parasitaemia in the population studied. The absence of high-density parasitaemia could be due to the regimen undertaken by the patients which have an anti-*Plasmodium* effect. The absence of high-density parasitaemia should however not be undermined, as HIV infection increases the risk of high-density malaria, which could lead to malaria.

Conclusions

This study revealed a high malaria co-infection and anaemia rates among HIV-infected patients in HAART clinics situated in FCT, Nigeria. The co-infection status further highlights the endemicity of *falciparum* malaria parasitaemia in the study region. The status of anaemia among co-infected patients was however not negatively affected. It is imperative to come up with strategies and plans to checkmate these ailments because of their public health significance. As seen in this study, RDTs, irrespective of the result obtained, need confirmation using gold-standard microscopy.

Study limitations

The major limitations of our study were our inability to conduct the recommended haemoglobin concentration tests to back up findings from the haematocrit (PCV) tests, and our inability to determine the patients' viral load. These were due to logistics reasons. We also had to obtain the patients' most recent CD4+ counts due to this setback. Finally, the study was a hospital-based cross-sectional survey.

Recommendations

Further studies capturing viral load determination and haemoglobin concentration tests are advocated. From our findings, we recommended that HIV-positive patients be routinely screened for malaria and anaemia before and in the course of antiretroviral therapy, as this would better inform the physicians on the best options for drug combination. A population study involving Abuja and its surrounding states is advocated, to determine the prevalence of Malaria co-infection and anaemia in the region. To further improve the establishment of guidelines for HIV management and prevention programmes, the government should include malaria and haemoglobin concentration tests among mainstream tests in health care delivery systems, to curb the spread of these epidemics. Cost of malaria prevention tools such as insecticides and LLIN should be subsidized for affordability by the average Nigerian. It would also help if dieticians were to be included in HIV-infected patients' treatment regimen to curb anaemia.

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Author Contributions

All the authors had access to the full dataset (including statistical reports and tables), and take responsibility for the integrity of the data and the accuracy of the data analysis. NCJA designed the study. PTS and DJO did the sampling. NCJA, PTS, DJO, and PA analysed the samples and interpreted the patient data regarding the co-infection and response to risk factors. NCJA and DJO were major contributors in writing the manuscript. All authors read and approved the final manuscript.

Availability of Supporting Data

The dataset used and/or analysed during the current study is available from the corresponding author on reasonable request.

Ethical Approval and Consent to Participate

Written informed consent was obtained from the participating adult patients and the guardians of the minors (patients < 18 years), and ethical clearance was obtained from the FCT Health Research Ethics Committee with the code FHREC/2019/01/22/23-03-2019. Additional approvals were also obtained from the local ethics committees of the selected medical centres. The clearance codes are as follows: FCTA/HHSS/GGH/GEN/05 (Gwarinpa General Hospital), FCTA/HHSS/HMB/GEN/038/T (Maitama District Hospital), and GHA/HR/GEN/0074/19 (Garki Hospital Abuja). Written Informed consent to participate in the study was obtained from the study participants before their being enrolled consecutively.

ORCID iD

Nneoma Confidence JeanStephanie Anyanwu https://orcid.org/0000-0003-1280-7605

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