The impact of HIV-associated immunosuppression on the Plasmodium falciparum chloroquine resistance transporter gene (PfCRT) of HIV patients in Akure, Nigeria

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Abstract

Background: The study evaluated the prevalence of malaria and Plasmodium falciparum chloroquine resistance transporter gene (PfCRT) in HIV patients attending Specialist Hospital, Akure. This study was carried out between April and June 2019. Three hundred and seventeen (317) patients attending the antiretroviral clinic (ART) were involved, out of which 89 (28.08%) were males and 228 (71.92%) were females. HIV test was done using the Unigold® HIV test kit, malaria test was done using thick and thin blood smear, CD4 test was done using the Partec® CD4 counter and PCR was used to detect the presence of plasmodium falciparum mutant gene. The data obtained from this analysis was subjected to Pearson’s Chi-square test.

Results: The overall result showed low prevalence of malaria (23.03%) in the sampled patients. Highest malaria prevalence (31.0%) was recorded in HIV patients with CD4 count between 200–500 cells/μl of blood, with the males recording 24.7% malaria prevalence. The age group 20–29 years recorded the highest prevalence of 27.3%. A higher prevalence 91.1% of PfCRT gene in HIV-positive and (40.0%) in HIV-negative patients was recorded with 100% prevalence in patients with CD4 count ≤ 200. This shows that the low prevalence of malaria recorded in this study could be credited to good health-seeking attitude of HIV patients and the upscale of HIV care and treatment centres.

Conclusion: The high prevalence of PfCRT gene shows that treatment of malaria with chloroquine is still being practised despite the availability of artemisinin-based combination therapy (ACTs) as the recommended regimen for malaria treatment.

Keywords: HIV, Malaria, PfCRT gene, CD4, Plasmodium falciparum

Background

Malaria and HIV are two of the most widely recognised and significant public health issues facing developing nations and the most well-known infection in sub-Saharan Africa (McInnes and Rushton 2010). These diseases establish the significant public health threat responsible for morbidity and mortality in Africa where half of the total populace is in danger of disease.

UNAIDS (2011) reported that in 2010, around 34 million individuals were living with HIV/AIDS of which 2.7 million are new infections, and the highest burden is in sub-Saharan Africa, where an expected populace of 22.9 million (68%) of HIV-infected individuals live (WHO 2011). The report additionally expressed that the
complete number of new HIV infections dropped by over 26% since 1997, when HIV infection pestilence was at the pinnacle (Piot and Quinn 2013). In Africa, an estimated 40 million individuals are infected with HIV, resulting in a yearly mortality of more than 3 million (McInnes and Rushton 2010), though more than 500 million clinical *P. falciparum* infections happen each year, with an excess of a million malaria-related cases (Okara et al. 2010). There is an impressive geographic overlap between the two sicknesses, especially in sub-Saharan Africa (Shott et al. 2012) and growing proof of an interactive pathology (Bousema and Drakeley, 2011). HIV has appeared to increase the danger of malaria infection and the advancement of clinical malaria sickness, with the best effect in insusceptible smothered people (Harries et al. 2010). On the other hand, malaria has appeared to induce HIV-1 replication in vitro (Gonzalez et al. 2012) and in vivo (de Ryckera et al. 2018). A natural clarification for these interactions lies in the cell-based invulnerable reactions to HIV and malaria fever (Olayemi et al. 2012).

Malaria is accounted for, to be able to up-control the HIV formative cycle by enhancing the movement of T cells as well as by inducing an intense pro-inflammatory cytokine drive, leading to arrival of operators, for example, TNF-α (Alemu et al. 2013). It has been recommended that this outcomes in the transient increase in plasma viral burden (James et al. 2018).

Until recently, chloroquine was the most broadly utilised medication to treat malaria fever. The spread of chloroquine resistance in Africa prompted an ascent in the malady trouble during the 1980s (White 2018), a difficulty that has tormented the area right up till today. Chloroquine acts by meddling with the detoxification of hematin, a lethal result of haemoglobin breakdown, in the *Plasmodium*’s digestive vacuole. A few molecular markers of *P. falciparum* drug resistance have been distinguished, including the *P. falciparum* CQ resistance transporter gene (*PfCRT*) related with chloroquine resistance, dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) genotypes related with sulfadoxine-pyrimethamine resistance. Previous investigations showed that *PfCRT* (K76T) mutation in *Plasmodium falciparum* can be utilised for surveillance of clinical CQ resistance (Haldar et al. 2018).

**Methods**

**Study area**

Akure is located in the rain forest zone between latitude 7° 15’ 0” N and longitude 5° 11’ 42” E. It has two major seasons: the rainy (wet) season that ranges from March to October and the dry season that ranges from November to February. The average annual rainfall is about 2378 mm, with temperature between 25.2 and 28.1 °C and relative humidity of 80% (Simon-Oke et al. 2018). The University of Medical Sciences Teaching Hospital Complex is located in Akure, the capital city of Ondo State, southwest of Nigeria.

**Selection criteria**

**Inclusion criteria**

HIV-infected patients placed on antiretroviral therapy (ART) when tested positive for HIV infection were included in this study.

**Exclusion criteria**

Patients who were HIV negative were excluded from the study.

**Study design**

This study was a case control hospital-based study consisting of both HIV-infected and non-infected patients attending the University of Medical Sciences Teaching Hospital Complex in Akure for treatment and counselling.

**Study population**

The study was conducted between April and June 2019, with 317 patients who were attending the University of Medical Sciences Teaching Hospital Complex in Akure for HIV treatment and counselling.

**Collection of blood sample**

A sterile multi-sample needle was used to collect about 2–3 ml of intravenous blood into an ethylenediaminetetraacetic acid (EDTA) sterile bottle to prevent coagulation of the blood samples.

**HIV test**

The disposable pipette found in the HIV test kit was used to take blood samples from EDTA bottles. Two drops of whole blood (approximately 60 μl) was added to the sample well of Unigold® Cassette kit. Two drops of the appropriate wash reagent were added into the sample well, and the results were read between 10 and 20 min (Unigold, 2017).

**CD4 count test**

Fifty microlitre whole blood was added into a Partec® test tube followed by the addition of 10 μl CD4 antibody. This was incubated in the dark between 10 and 15 min at room temperature. It was mixed for 5 min with a 230-volt vortex mixer. Eight hundred fifty microlitre CD4 buffer solution (no lyse dilution buffer) was added, incubated in the dark between 10 and 15 min and mixed gently by the same vortex mixer for 5 s. The mixed sample was plugged into a counter machine for CD4+ count reading (Renault et al., 2010).
Malaria microscopic test
With the use of Pasteur pipette, 2–3 drops of blood in the EDTA bottle were taken and placed on a well-labelled clean grease-free glass slide. The blood on the slide was allowed to dry after which the film was stained with 10% Giemsa stain and allowed to dry for 10 min. After 15 min, the stain was washed off rapidly with distilled water and allowed to dry. One drop of immersion oil was added to the film and then viewed under the microscope at × 100 objective lens for characteristic features of malaria parasite (Krafts et al. 2011).

Sample collection for molecular analysis
With the use of Pasteur pipette, 3 drops of blood was spotted on a 3-mm Whatman® filter paper inscribed between a cardboard paper (on which proper labelling has been done) and allowed to dry under room temperature until the blood turns brown.

Sample preservation
The Whatman® filter paper on which the blood samples have been spotted to dry were placed in a transparent Ziploc bag in which desiccants were added to prevent reaction with moisture. The Ziploc bag was placed in a refrigerator after which it was transported to the Molecular Laboratory of the Department of Biochemistry, Federal University of Technology Akure (FUTA), for molecular analysis.

DNA extraction, amplification of \( PfCRT \) gene fragment and agar gel electrophoresis were carried out according to standard procedures (Jean Bioscience 2015; James et al. 2018).

Statistical analysis
The statistical parameters for the analysis of data were subjected to Pearson’s Chi-Square Test (Table 1). Charts and tables were created using the Microsoft Excel software application.

Results
Prevalence of malaria parasite according to HIV status
A total number of 317 blood samples were collected from already confirmed HIV-positive patients attending the hospital, of which 73 (23.03%) patients tested positive for the presence of malaria parasite while 244 (76.97%) tested negative. Eighty-nine (28.08%) patients were males and 228 (71.92%) were females. Eighteen (5.68%) were ≤ 20 years, 11 (3.47%) were between 20 and 29 years, 113 (35.65%) were between 30 and 39 years, 94 (29.65%) were between 40 and 49 years and 81 (25.55%) were ≥ 50 years (Fig. 1).

Prevalence of malaria parasite in relation to CD4 cell count
Of the 317 patients, 38 (11.99%) had CD4 count ≤ 200 cells/μl of blood, and 2 (5.3%) tested positive for malaria parasite. One hundred forty-five (45.74%) had CD4 count between 200 and 500 cells/μl, and 45 (31.0%) tested positive. One hundred thirty-four (42.27%) had CD4 count ≥ 500 cells/μl, and 26 (19.4%) tested positive. There was significant difference in the prevalence of malaria parasite according to HIV status and CD4 cell count (\( P < 0.05 \)) (Table 2).

Prevalence of malaria parasite according to gender and age
Out of 89 male patients, 22 (22.4%) tested positive for malaria parasite, and of the 228 female patients, 51 (24.7%) tested positive for malaria. There was no significant difference in the prevalence of malaria according to gender, \( P = 0.66 \) \((P > 0.05)\). Eighteen (5.68%) were ≤ 20 years, and 2 (11.1%) tested positive for malaria. Eleven (3.47%) were between 20 and 29 years, and 3 (27.3%) were positive. Of the 30–39, 40–49 and ≥ 50 age ranges, 21 (18.6%), 25 (26.6%) and 22 (27.2%) were positive for malaria parasite, respectively. There is no significant difference in the prevalence of malaria according to age \( P = 0.37 \) \((P > 0.05)\) (Table 3).

Fifty (50) blood samples were spotted and tested for the presence of the mutant \( PfCRT \) gene (Plate 1), out of which 43 (86%) tested positive. Two (4%) with CD4 count ≤ 200 cells/μl of blood tested positive for the gene. Twenty-seven (87.1%) with CD4 count between 200 and 500 cells/μl of blood tested positive for the gene. Fourteen (82.4%) with CD4 count ≥ 500 cells/μl of blood tested positive for the gene. Statistical analysis showed that there is no significant difference in the prevalence of \( PfCRT \) among the patients, \( P = 0.77 \) \((P > 0.05)\) (Table 3).

Out of the 17 (34%) and 29 (87.9%) males and females, 14 (82.4%) and 29 (87.9%) tested positive for the gene, respectively. There was no significant difference in the

| PCR round | Primer | Primer sequence | Time  |
|-----------|--------|-----------------|-------|
| 1st round | Crtd-A (forward primer) | 5′-GTTTGGAGGTTCTTGCTTGGTA-3′ | 57.5 °C |
|           | Crtd-B (reverse primer) | 5′-GACCTATGAGGCACAAATGACTG-3′ | 56.7 °C |
| 2nd round | Crtd-C (forward primer) | 5′-TGTGCTCTAGTGTTAACAAC-3′ | 50.0 °C |
|           | Crtd-D (reverse primer) | 5′-CAAACACTATAGTACCAATTT-3′ | 46.1 °C |

Table 1 Sequence of primers
prevalence of PfCRT gene according to gender, \( P = 0.59 \) \((P > 0.05)\) (Table 4).

Age groups \( \leq 10 \) years, 21–30 years and 71–80 years recorded the highest prevalence (100%) while the lowest prevalence (0%) was recorded for age groups 11–20 years and \( \geq 80 \) years. There was no significant difference in the prevalence of PfCRT according to age \( P = 0.57 \) \((P > 0.05)\) (Table 5).

**Discussion**

HIV/AIDs infected patients are at high risk of malaria infection and clinical diseases in endemic areas, as a result of their weakened immune response. The overall prevalence of malaria infection among HIV-infected patients was 23.03%. The low prevalence recorded in this study could be credited to the good and encouraging health-seeking attitude of HIV patients, the upscale of HIV care and treatment centres and thus, the better accessibility to health care services, as there is prompt health care for HIV-infected patients (Berg et al. 2014). Other factors that could be liable for the low prevalence of malaria in this study area are the use of preventive strategies such as insecticide treated nets (ITNs), which are distributed freely and at regular intervals in the study area (Iliyasu et al. 2013; Berg et al. 2014). Also, most of the patients recruited for this study had been placed on antiretroviral therapy (ART) when they tested positive for HIV, which has been prescribed for all HIV-infected patients in sub-Saharan Africa and has been discovered to decrease records of malaria sickness (Sanne et al., 2012).

Malaria prevalence was higher (31%) in patients with CD4 count between 200 and 500 cells/\( \mu \)l of blood which agreed with Iliyasu et al. (2013). The result of this research is in agreement with Kasirye et al. (2016) who revealed that malaria episode did not differ by CD4 count at ART commencement, enrolment or during follow-up. This can be associated to the well-established foundation that low CD4 T lymphocytes cells are related with a higher danger of opportunistic infections and rapid disease progression and vice versa (Geldmacher and Koup 2012). CD4 T cells responsible for protection against malaria are built up over time as an effect of recurrent malaria attacks but are destroyed in HIV-infected individuals, thus leading to increased clinical malaria. The major target of HIV is the CD4 T cells which the virus attacks and destroys, thereby resulting in gradual
immune decline from the normal range of 500–1500 cells/µl of blood to 200–500 cells/µl (Février et al. 2011). The PfCRT prevalence of 91.1% recorded in this study indicates the presence of the mutant gene and widely spread in Nigeria. This is similar to the previous reports of Okungbowa and Mordi (2013) and Balogun et al. (2016). This may have been facilitated by the migration of people having the resistant gene from one place to another for various activities (Okungbowa and Mordi 2013), indiscriminate use of drugs (drugs abuse) for treatment of malaria and easy access to over-the-counter malaria drugs without appropriate diagnosis and prescription in people living with HIV, long time use of chloroquine as antimalarial drug and constant exposure of the parasite to drugs that could lead to development of the resistant gene (Muhammad et al. 2017).

Patients with CD4 ≥ 500 cells/µl had the highest PfCRT prevalence of 92.9%, which could be as a result of the patients involved in this study are dedicated to a regular and highly monitored regime of the highly active antiretroviral therapy (HAART) which helped increase the CD4+ count of the patients. This explained why the results showed no significant relationship between low CD4+ T cell count and increased parasitaemia in these patients.

Patients under the age groups ≤ 10, 21–30 and 71–80 years showed the highest PfCRT prevalence (100%). For age group ≤ 10, it could be as a result of immature immune organs. The older age groups are independent adults who have the liberty to take drugs at will, and also young patients who are under the authority of parents or guardians and have been exposed to the various over-the-counter and antimalarial drugs such as chloroquine.

There was no significant difference (p = 0.31) between the prevalence of PfCRT gene according to HIV status and age. This agreed with Wurtz et al. (2012) in Senegal, which also found no significant difference between the gene point mutations and age of patients because most of the positive samples in that study were from patients aged 22–40 years, and this limited range of age may serve as a constraint when examining such association.

Mutant PfCRT genes were dominant in middle-aged female patients than male patients. This disagrees with the report of Simon-Oke et al. (2018) which reported the predominance of mutant PfCRT in middle-aged men who took chloroquine. The presence of mutant Pfcr genes among the subjects indicates that mutant Pfcr gene is widely spread in the study area.

**Conclusion**
The high prevalence of mutant PfCRT gene is a key indicator of Plasmodium falciparum chloroquine resistance spread in patients attending the study area. Self-treatment with chloroquine and other over-the-counter drugs are still promptly accessible in the market because of their affordability, accessibility and low implementation of the malaria treatment rules

### Table 3
Prevalence of PfCRT gene according to CD4 cell count

| CD4 cell count | N = 50 | PfCRT prevalence N = 43 (%) | χ² | Df | P value |
|----------------|-------|-----------------------------|----|----|---------|
| ≤ 200          | 2     | 2 (100)                     | 0.54 | 2 | 0.77    |
| 200–500        | 31    | 27 (87.1)                   |     |    |         |
| ≥ 500          | 17    | 14 (82.4)                   |     |    |         |

(P > 0.05)

### Table 4
Prevalence of PfCRT gene according to gender

| Gender | N = 50 | PfCRT prevalence N = 43 (%) | χ² | Df | P value |
|--------|-------|-----------------------------|----|----|---------|
| Male   | 17    | 14 (82.4)                   | 0.29 | 1 | 0.59    |
| Female | 33    | 29 (87.9)                   |     |    |         |

(P > 0.05)
that recommends the use of artemisinin combination therapy (ACTs). The partner drugs to ACTs are also threatened by the development of resistance if treatment of malaria with antimalarial monotherapy is not established. HIV infection can lower the efficacy of antimalarial treatment, further buttressing the need for patients’ education on the need for early diagnosis and prompt treatment of malaria. Physicians and other health care workers should adhere to standardised clinical guidelines for managing HIV-malaria co-infection.

Abbreviations
HIV: Human immunodeficiency virus; AIDS: Acquired immunodeficiency virus; TNF: Tumour necrosis factor; CQ: Chloroquine; DNA: Deoxyribonucleic acid; CD4: Cluster of differentiation 4

Acknowledgements
The authors thank all the HIV patients who agreed to be part of the study, as well as the management and staff of the State Specialist Hospital, Akure.

Authors’ contributions
IAS and FO designed the work; IAS and AOA carried out the field and laboratory experiments; IAS, FO and AOA compiled, wrote, and approved the final manuscript.

Funding
Not applicable

Availability of data and materials
Not applicable

Competing interest
The authors declare that there is no competing interest whatsoever.

Ethics approval and consent to participate
The research proposal was submitted to Health Research Ethics Committee (HREC) of the Ondo State Ministry of Health for approval. The Committee approved the research as ethically cleared after thorough consideration of the research proposal. Approval was also gotten from the Physician-in-Charge (PIC) of the University of Medical Sciences Teaching Hospital Complex, Akure. Consent forms were signed by the study participants.

Consent for publication
Not applicable

Table 5
Prevalence of PfCRT gene in relation to age

| Age     | N = 50 | PfCRT prevalence N = 43 (%) | X² | DF | P value |
|---------|--------|----------------------------|-----|----|---------|
| ≤ 10    |        | 1 (100.00)                 | 3.00| 4  | 0.57    |
| 11–20   |        | 0 (0)                      |     |    |         |
| 21–30   |        | 3 (100.00)                 |     |    |         |
| 31–40   | 14     | 13 (92.86)                 |     |    |         |
| 41–50   | 13     | 11 (84.62)                 |     |    |         |
| 51–60   | 10     | 9 (90.00)                  |     |    |         |
| 61–70   | 7      | 5 (71.43)                  |     |    |         |
| 71–80   | 1      | 1 (100.00)                 |     |    |         |
| ≥ 80    | 0      | 0 (0)                      |     |    |         |

(P > 0.05)

Received: 12 June 2020 Accepted: 17 August 2020
Published online: 14 September 2020

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