Quest Journals
Journal of Medical and Dental Science Research
Volume 8~ Issue 8 (2021) pp: 31-34
ISSN(Online) : 2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

Prevalence of Multi-Drug Resistance Gene of *Plasmodium falciparum*In Keffi Nigeria.

Olayonwa Damilare Ezekiel[1*], AkeleJide Emmanuel [2], MonsuruMutiu Sunday[3], Ode Ebenizer Oluwasola[4]

[1]*Research assistant, Unique Medical and Research Laboratory*
[2]*Research supervisor, Unique Medical and Research Laboratory*
[3]*Medical laboratory scientist General Hospital Maiyama*
[4]*Medical laboratory scientist Okoloba Hospital*
[*]*Corresponding Author: Olayonwa Damilare Ezekiel.*

ABSTRACT:*The main factor holding back malaria elimination strategies is the continuous emergence of Plasmodium falciparum parasites resistant to malaria drugs. After the ban of chloroquine in 2005, Artemisinin-based combination therapies (ACT) recommended by WHO has become the first line treatment drug for malaria in Nigeria. However, evidence has emerged on the risk of developing resistance to ACT, thereby leading this research work to aimed at determining the prevalence of P. falciparum chloroquine resistance transporter gene (Pfcrt) and P. falciparum multidrug resistance gene 1 (Pfmdr-1) in keffi between year 2019 to 2021. There was no significant increase in the prevalence of Pfcrt gene CVMNK wild type in period of 2019-2020 (25% to 25.3%) and in 2020-2021 (25.3% to 26.4). The prevalence of P. falciparum infections carrying N86 was low in 2019 to 2020 (24%-26%), and significantly increased from 26% in 2020 to 68% in 2021. The prevalence of P. falciparum infections carrying 184F reasonably increased from 34% in 2019 to 47% in 2020, and also significantly increased from 47% in 2020 to 81% in 2021. There was no significant difference in the prevalence of P. falciparum infections carrying D1246 in 2019 to 2020 and 2020 to 2021. The speedy changes observed in Pfmdr-1gene may be as a result of stoppage in the use of artemisinin-based monotherapies (including Chloroquine) and widely use of Artemisinin-based combination therapies (ACT), therefore there is need to develop and explore more on the molecular markers of Plasmodium falciparum to malaria drugs resistance.*

KEY WORDS: *Plasmodium falciparum, Artemisinin, gene, Polymorphism, Chloroquen.*

Received 10August, 2021; Revised: 24 August, 2021; Accepted 26 August, 2021 © The author(s) 2021. Published with open access at www.questjournals.org

I. INTRODUCTION

Malaria, the life-threatening vector-borne disease, remains a global threat and a major public health issue across the world [1]. About 219 million cases and 435,000 deaths from malaria occurred worldwide in 2017 [2]. The Major factor hindering malaria elimination strategies is the continuous emergence of *Plasmodium falciparum* parasites resistant to all known existing malaria drugs [3].

Antimalarial drug resistance may occur due to variations in the build-up of drug or efflux mechanisms (as in the case of chloroquine, amodiaquine, quinine, halofantrine, and mefloquine resistance) or due to diminished affinity of the drug's target, which may result from point mutations in the respective genes that encode these targets (pyrimethamine, cycloguanil, sulphonamide, atovaquone, and artemisinin resistance) [4, 15, 16]. Following the resistance of *Plasmodium falciparum* to antimalarial drugs such as chloroquine and sulfadoxine-pyrimethamine, the WHO in 2001 endorsed artemisinin combination therapies (ACTs) comprising drug combinations of artemether-lumefantrine (AL) and amodiaquine-artesunate (AQ-AS) as first-line treatment for uncomplicated malaria[5, 17].

Nigeria banned Chloroquine in 2005 and Artemisinin-based combination therapies (ACT) recommended by WHO has become the first line treatment drug for malaria[6]. However, evidence has emerged on the risk of developing resistance to ACT [7]. Many studies has been conducted on multi-drug resistance gene of Plasmodium *falciparum* in deferent part of Nigeria, butthere is no report or study of prevalence of multi-drug resistance gene of Plasmodium *falciparum* inKeffi, as Keffi is entirely another geographical are in Nigeria.

Therefore this research work was aimed to determine the prevalence of P. *falciparum* chloroquine resistance transporter gene (Pfcrt) and P. *falciparum* multidrug resistance gene 1 (Pfmdr-1) in Keffi between year 2019 to 2021. To achieve this aim, Diagnosis of P.*falciparum* was done on the samples collected, P. *falciparum* chloroquine resistance transporter gene (Pfcrt) at codons 72-76 and P. *falciparum* multidrug resistance gene 1 (Pfmdr-1) at codons 86, 184, and 1246 was amplified, also Single Nucleotide Polymorphism (SNP) of the Amplified genes at codons 72-76 and codons 86, 184, and 1246 was determined.

II. MATERIALS AND METHOD

Sample Population: Sample population of this study comprised of patients that were under malaria treatment in Federal Medical Centre Keffi, Nigeria. The targeted patients were those that were newly placed on Artemisinin-based combination therapies (ACT) and those that were about to be placed on ACT. Approval for the study was obtained from Ethical and Research Committee of Federal Medical Centre, Keffi.

Sample Collection:500 samples were collected in total for the study; 160 in November and December 2019, 165 in April and May 2020, 175 in February and March 2021 frompatients that were newly placed on Artemisinin-based combination therapies (ACT) and those that were about to be placed on ACT atFederal Medical Centre, Keffi.The patients were screened for P. *falciparum* and the initial screening for P. *falciparum* was conducted using microscopic observation of thin and thick Giemsa-stained blood films and those who tested positive for P. *falciparum* were recruited in the study. 1 ml of venous blood was collected into vacutainer tubes containing EDTA, a sub-sample of this was spotted onto Whatman No 3 filter paper for DNA extraction.

DNA Extraction:DNA extraction was done usingNucleoSpin Genomic DNA Bloodpure Kit a commercially prepared kit. (Macherey-Nagel, Du¨ ren, Germany). Extraction was done based on manufacturer's instructions.

Polymerase Chain Reaction and SNP analysis of Pfcrt and Pfmdr-1genes: Outer and nested PCR was performed on the extracted DNA to amplified Pfmdr-1 gene at 86, 184, and 1246 and Pfcrt gene at condo 72-76 as described by [8].

The concentration of the primary mixture for amplification was 1× PCR buffer, 2mM MgCl2, 125 µM dNTP, 25 nM of each primer (F/R), and 1X dream Taq green DNA polymerase. One microliters of DNA template was added to a reaction volume of 19µL. The secondary amplifications was done in a 20µL reaction volume containing 1× PCR buffer, 1mM MgCl2, 125µM dNTP, 125 nM of each primer (F/R), and 1X dream Taq green DNA polymerase (Hassan *et al*., 2016). The product of the first amplification was used as the template for the second PCR (1µL/reaction)[8].

Sequence-specific oligonucleotide probe (SSOP)–enzyme-linked immunosorbent assay as described by [9] was used to determine Pfcrt Single Nucleotide polymorphism at codons 72-76. Pfmdr-1 SNP was determined at codons 86, 184, and 1246 using restriction fragment length polymorphism as described by [10].

Statistical Analysis: A statistical package for social sciences (SPSS) 23.0 versions was used for the analysis of the data appropriately. Pearson's chi squared test was use to determined differences in frequency and Fishers exact test was also used.The level of significance was taken at 95% confidence intervaland $P<0.05$ was considered significant.

III. RESULTS

The test results indicated that 85(53.1%) out of 160, 76(46.1%) out of 165, 102(58%) out of 175 samples collceted were tested positive to P. *falciparum*for years 2019, 2020 and 2021 respectively.

The frequency ofPfcrt geneCVMNK wild type which include mixed CVMNK/CVIET infections as against mutant CVIET haplotype infections showed a significant increase of CVMNK haplotype from 39.3% in 2019 to 57.5% in 2020 ($x^2 = 12.2$, $P = 0.003$), and 57.5% in 2020 to 70.4% in 2021 ($x^2 = 14.4$, $P = 0.004$)

The prevalence of Pfcrt gene CVMNK wild type including mixed CVMNK/CVIET infections versus pure mutant CVIET haplotype infections increased significantly from 34.2% in 2019 to 52.3% in 2020 ($x^2 = 10.2$, $P = 0.002$) and 52.3% in2020 to 75.8% in 2021 ($x^2 = 9.02$, $P = 0.005$).

The temporal prevalence of SNPs of Pfmdr-1 gene at codons 86, 184, and 1246 was analyzed. Temporal changes in codon 86 SNP distribution of P. *falciparum* infections carrying N86 wild types, including mixed N86/86Y infections, versus single mutant-type 86Y infections. The prevalence of P. *falciparum* infections carrying N86 wild type was low in 2019 to 2020 (24%-26%), while the prevalence of P. *falciparum* infections carrying N86 significantly increased from 26% in 2020 to 68% in 2021($x^2 = 14.3$, $P = 0.005$).

The prevalence of P. *falciparum* infections carrying 184F including mixed 184F/184Y infections, versus single mutant-type 184Y infections reasonably increased from 34% in 2019 to 47% in 2020($x^2 = 5.52$, $P = 0.048$), and 47% in 2020 to 81% in 2021($x^2 = 12.9$, $P = 0.004$). There was no significant difference in the prevalence of P.

falciparum infections carrying D1246 in 2019 to 2020 and 2020 to 2021 ($x^2 = 0.136$, P = 0.775 and $x^2 = 0.248$, P = 0.512 respectively).

Mixed genotype of Pfmdr-1 gene was omitted from examination of temporal changes in frequency of SNPs. The frequency of the N86 wild type significantly increased from 45.5% in 2019 to 52.4% in 2020 ($x^2 = 7.52$, P = 0.004), and significantly increased from 52.4% in 2020 to 78.4% in 2021($x^2 = 6.23$, P = 0.003). The frequency of the 184F wild type reasonably increased from 20.2% in 2019 to 32.5% in 2020 ($x^2 = 3.04$, P = 0.220), and also did not increased more than 35.7% in 2021($x^2 = 2.12$, P = 0.152). No significant differences in frequency of the D1246 wild type 21% in 2019 to 25.6% in 2020 ($x^2 = 3.23$, P = 0.042) and 25.6% in 2020 to 30.5% in 2021 ($x^2 = 2.42$, P = 0.035)

IV. DISCUSSION

Nigeria stopped the use of artemisinin-based monotherapies most especially Chloroquine in 2005, and artemether-lumefantrine combine drug become widely used[6]. There is significant increase in Pfcrt gene in Keffi, even at the fading out of chloroquine in Nigeria which suggested that there is illegal use of chloroquine in Nigeria. Though the study was carried out during the COVID-19 Pandemic, and there were several reports of people using chloroquine as a prophylaxis, this might have contributed greatly to increase in Pfcrt gene. This finding is similar to the report of the sturdy conducted in Mozambique that indicated an increase in the Pfcrt gene and also suggested that it might be as a result of low price and easy access of chloroquine in Mozambique[10].

Despite unauthorized use of chloroquine, Artemisinin-based combination therapies (ACT) is widely use in the country. There have been reports of increase in the prevalence of Pfmdr-1 N86 wild type in some part of Nigeria which was associated to the wide spread of the use artemether-lumefantrine combine drug[11]. Theprevalence of Pfmdr-1 N86 wild type was low in Keffi between the year of 2019 and 2020, while it significantly increased from 26% in 2020 to 68% in 2021. The sudden increase in the prevalence of Pfmdr-1 N86 wild type in 2021 might be associated to more increase in the use of artemether-lumefantrine combine drug.

There was an increase in the prevalence of Pfmdr-1 184F mutant-type from 34% in 2019 to 47% in 2020 and also significantly increased from 47% in 2020 to 81% in 2021. This might be as a result of increase in the resistance of chloroquine by P. *falciparum*as associated by some report[12, 13]. Several studies on prevalence of SNPs of Pfmdr-1 gene at codons 184 have shown that P. *falciparum*carrying Y184 is mostly found to East and Central Africa while the mutant 184F is mostly found in West Africawhich validates our findings [11, 12].

There was no significant difference in the prevalence of P. *falciparum* infections carrying D1246 in 2019 to 2020 and 2020 to 2021, and this agrees with studies done in some part of Nigeria.

V. CONCLUSION

The speedy changes observed in prevalence of SNPs of Pfmdr-1 gene at codons 86, 184, and 1246 may be as a result of stoppage in the use of artemisinin-based monotherapies (including Chloroquine) and widely use of Artemisinin-based combination therapies (ACT), therefore there is need to develop and explore more on the molecular markers of Plasmodium *falciparum* to malaria drugs resistance. There should be regulation of the use of antimalarial drugs, most especially chloroquine as it is still easily accessible by people despite the ban.

ACKNOWLEDGEMENT

Authors will like to acknowledge the staff of Unique medical and research Laboratory, and also Staffs of Federal medical center Keffi for their corporation during the sample collection process

REFERENCES

[1]. World Health Organization, "2015 World malaria report," World Helath Organization, 2018. [Online]. Available: http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/.. [Accessed 15 December 2019].
[2]. R. Diji-geske, G. Olasehinde, I. Fadina, D. Arogundade and P. Derby, "Epidemilogical data of *falciparum* malaria in Ado-oda/Ota Southwest Ogun State Nigeria," *Journal of malaria parasite*, vol. 19, pp. 1398-1402, 2018.
[3]. G. I. Olasehinde, D. O. Ojurongbe, O. J. Akinjogunla, J. O. Egwari and A. O. Adeyeba, "Prevalence of malaria and predisposing factors to antimalaria drug resistance in Southwestern Nigeria," *Resistance Journal of Parasitology*, vol. 3, pp. 92-101, 2015.
[4]. S. Shewta, M. Bikash and S. Rakesh, "Challenges of drug resistant malaria," *Journal of Parasite*, vol. 21, pp. 61-65, 2014.
[5]. World Health Organization, "Status Report on Artemisinin resistance and ACT efficiency," WHO, 2018. [Online]. Available: http:// www.who.int/Status Report on Artemisinin resistance and ACT efficiency/report/en/. [Accessed 15 December 2019].
[6]. G. I. Olasehide, "In-vitro and Molecular studies on resistance P.*falciparum* to antimalaria drug in Ogun state, Southwestern Nigeria," *Journal of Microbiology*, vol. 13, pp. 67-76, 2010.
[7]. M. J. Mackinnon, "Drug resistance Model of Malaria," *Acta Tropica*, vol. 94, pp. 207-217, 2005.
[8]. A. O. Hassan, O. G. Ademujimi and A. O. Popoola, "Molecuar screening of S769N mutation for PFATPASE in subjects with Plasmodiasis in Keffi," *International Journal of Medicine and Biomedical Research*, vol. 5, no. 1, pp. 28-34, 2016.

[9]. T. . T. Thomas, B. M. Laura, H. H. Helle, V. E. Elsa , C. Derek, B. Ib C and A. Michael, "Rapid Selection of Plasmodium *falciparum* Chloroquine Resistance Transporter Gene and Multidrug Resistance Gene-1 Haplotypes Associated with Past Chloroquine and Present Artemether-Lumefantrine Use in Inhambane District, Southern Mozambique," *America Journal of Tropical Medicine and Hygiene*, vol. 88, no. 3, pp. 536-541, 2013.

[10]. M. Alifrangis , S. Enosse, R. Pearce, C. Drakeley , C. Roper, I. F. Khalil, W. M. Nkya, A. M. Ronn, T. G. Theander and I. C. Bygbjerg, "A simple high-throughput method to detect Plasmodium *falciparum* single nucleotide polymorphisms in the dihydrofolate reductase, dihydropteroate synthase, and P. *falciparum* cloroquine resistance transporter genes using ploymerase chain reaction and enzyme," *American Journal of Tropical Medicine and hygiene*, vol. 72, pp. 155-162, 2005.

[11]. A. Auwal, S. Mahmoud, M. Hauwa, Y. Bassa, I. Mohammed, B. Emmanuel, S. Takaya, I. Daniel, K. Kiyoshi, H. kenji, C. Richard and S. Mohammed, "Plasmodium *falciparum* multidrug resistance gene-1 polymorphisims in Northern Nigeria: Implications for the Continued Use of artemether-lumefantrine in the region," *Malaria Journal*, vol. 19, pp. 2-10, 2020.

[12]. S. J. Foote, D. E. Kyle, R. K. Martin, A. Oduola, K. Forstyth and D. J. Kemp, "Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in plasmodium *falciparum*," *Nature*, vol. 345, pp. 255-258, 1990.

[13]. P. E. Ferrira, G. Holmgren, M. I. veiga, P. Uhlen and A. Kaneko, "PFMDR1: mechanism of transport modulation by functional polymorphism," *PLoS One*, vol. 6, p. 238, 2007.

[14]. C. T. Happi, G. O. Ghotosho, O. A. Folarin, A. Sowunmi, T. Hudson and M. O'Neil, "Selection of Plasmodium *Falciparum* multidrug resistance gene 1 alleles in asexual stages and gametocytes by arthemither-lumefatnterine in nigeria children with uncomlicated *falciparum* malaria.," *Antimicrob Agents Chemother*, vol. 53, pp. 888-895, 2009.

[15]. N. White, "Antimalaria drug resistance and combination chemotheraphy," *Bio Sci*, vol. 354, pp. 739-749, 1999.

[16]. P. Cravo, H. Napolitano and R. Culleton, "How genomics is contributing to the fight against artemisinin-resistance malaria parasite," *Acta Trop*, vol. 148, pp. 1-7, 2015.

[17]. S. J. Reiling and P. Rohrbach, "Monitoring PfMDR1 transport in Plasmodium *falciparum*," *Malaria Journal*, vol. 14, pp. 251-261, 2015.