MOLECULAR CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* RESISTANT GENES TO CHLOROQUINE AND SULPHADOXINE-PYRIMETHAMINE IN CHILDREN WITH UNCOMPLICATED MALARIA IN LAGOS

A thesis submitted to the School of Postgraduate Studies,
University of Lagos, Nigeria in partial fulfillment of the requirement for the award of Doctor of Philosophy (Ph.D.)
Degree in Medical Parasitology

BY

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UNIVERSITY OF LAGOS
SCHOOL OF POST GRADUATE STUDIES
CERTIFICATION

This is to certify that the thesis:

"Molecular Characterization of *Plasmodium falciparum* Resistant Genes to Chloroquine and Sulphadoxine-Pyrimethamine in Children with Uncomplicated Malaria in Lagos"

Submitted to the school of Postgraduate Studies, University of Lagos

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DOCTOR OF PHILOSOPHY (Ph.D.)

is a record of original research work carried out

By

OLADOSU, OLADIPO OLARINRE

in the Department of Medical Microbiology and Parasitology

DECLARATION

MOLECULAR CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* RESISTANT GENES TO CHLOROQUINE AND SULPHADOXINE-PYRIMETHAMINE IN CHILDREN WITH UNCOMPLICATED MALARIA IN LAGOS

BY

OLADOSU, OLADIPO OLARINRE

THIS IS TO CERTIFY THAT THIS RESEARCH WORK WAS CARRIED OUT

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DEDICATION

This thesis is dedicated to God Almighty, my Parents (Deacon and Mrs .J. Oladosu), my siblings and my wife.

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ABSTRACT

Malaria is one of the major causes of morbidity and mortality in young children in sub-Saharan Africa where it presents primarily with fever. The reports of malaria prevalence in Nigerian children are divergent with wide variation in prevalence reports despite the scaling-up of malaria control in many countries, including Nigeria. Despite the change in the National antimalarial treatment policy from Chloroquine (CQ) and Sulphadoxine-pyrimethamine (SP) to artemisinin combination therapies (ACTs), CQ and SP are still frequently used in health facilities in the general population because it is cheap, affordable and accessible. Resistance to CQ and SP has contributed to increase mortality caused by *Plasmodium falciparum* infections. The continuous use of non-efficacious antimalarials will consequently result in an increase in antimalarial- resistant *Plasmodium* parasites which could pose a threat to the partner drugs to the Artemisinin such as (Amodiaquine, Mefloquine etc). Genetic markers to predict *Plasmodium* parasites' resistance especially for single nucleotide polymorphism (SNPs) have the potential to be employed in an integrated fashion to provide timely information that is useful to policy makers on *P falciparum* resistance to antimalaria. Children less than 12years old, who presented with documented fever or history of fever in the last 24 hours between July 2007 and April 2008 were enrolled in this study. Of the 1211 children (<12years) enrolled, 251(20.7%) were slide positive for malaria parasites. Children in the age groups 0-≤1 and >1-12years had a prevalence of 11% and 5.8% respectively (P=0.001). While children in the age group 0-≤5 and >5-12years had malaria prevalence of 16.9% and 42.1% respectively (P=0.001). Of the malaria positive children 33.9% had parasitaemia of less than 500p/µl (P=0.001). This indicated a shift in malaria prevalence from the usually reported 0-≤5years to the >5-12years old children. The occurrence of point mutations and haplotypes were investigated in DNA obtained from blood samples of slide positive children by assaying for *Plasmodium falciparum* chloroquine resistance transporter (*Pfcrt*) and *Plasmodium falciparum* multi-drug resistance (*Pfmdr1*) genes associated with CQ and other 4-aminoquinolines resistance. The frequency of the mutant *Pfcrt* haplotype, CVIET, was 91.6% while, the frequency of *Pfmdr1* in the positive microscopy samples was

62.2% and 69.0% for codon Y86 and F184 respectively. No mutation was seen at codons 1034, 1042 and 1246 of the *Pfmdr1* genes in this study. The combined *Pfcrt* and *Pfmdr1* haplotypes showed that most of the malaria positive children had CVIET + YFSND haplotypes (61.9%). There was no association between parasitaemia and *Pfcrt* and *Pfmdr1* haplotypes. The high prevalence of the mutant genes (CVIET) seen among this children could limit the already poor efficacy of the partner drugs to the Artemisinin. Thus, the continuous use of CQ and other 4-aminoquinolines when used as monotherapy in Lagos State could increase the frequency of mutations in *Pfcrt* and *Pfmdr1* genes. Sequenced results of the amplified *Dihydrofolate reductase* (*Dhfr*) and *Dihydropteroate synthase* (*Dhps*) genes for SNPs showed mutations at codons 108 (96.5%), 59 (92.9%) and 51 (94.7%). Four *Dhfr* haplotypes (ACIRNVI, ACICNVI, ACNCNVI and ACNCSVI) and eleven *Dhps* haplotypes (ISGKAA, VAGKGS, VAGKAA, IAGKAS, ISAKAA, IAAKAA, ISGKGA, IFGKAS, IAGKAA, VSGKGS and ISGKAS) were grouped. The grouped data showed that most of the isolates (92.9%) had the triple *Dhfr* mutation (51I, R59 and 108N), while the majority of the isolated *P. falciparum* in the *Dhps* gene had mutation at codon G437 (96.6%). The combined haplotypes assessment showed that ACIRNVI + ISGKAA (quadruple mutation) had the highest occurrence (56.7%). There was no mutation at V16, R50 and L164 of the *Dhfr* gene and at E540 of the *Dhps* gene. These reports showed a high frequency of mutations in *Dhfr* and *Dhps* genes in Nigeria. Furthermore, it provided information on haplotypes and its distribution on clinical samples from children in Lagos. The continuous use of SP as monotherapy against malaria and the reported high frequency of mutations in *Dhfr* and *Dhps* genes could compromise the efficacy of SP-artesunate combination.