POLYMORPHISMS IN PLASMODIUM FALCIPARUM *PfAMA1* AND *PfRH5* GENES IN ISOLATES FROM SELECTED CENTRES IN LAGOS, NIGERIA.

by

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DECLARATION

We hereby declare that this thesis titled "Polymorphisms in *Plasmodium falciparumPfAMA1* and*PfRH5* Genes in Isolates from Selected Centres in Lagos, Nigeria" submitted to the School of Postgraduate Studies, University of Lagos, Nigeria for the award of Doctor of Philosophy in Biochemistry is a record of original research work carried out by **AJIBAYE,**Olusolain Biochemistry, Department of Biochemistry, Faculty of Basic Medical Sciences,College of Medicine, University of Lagos, Nigeria.

The work has not been submitted previously in whole or in part to qualify for any academic award.

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UNIVERSITY OF LAGOS
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CERTIFICATION

This is to certify that the Thesis:

POLYMORPHISMS IN PLASMODIUM FALCIPARUM *PfAMA1* AND *PfRH5* GENES IN ISOLATES FROM SELECTED CENTRES IN LAGOS, NIGERIA

Submitted to theSchool of Postgraduate Studies, University of Lagos

For the award of the degree of

DOCTOR OF PHILOSOPHY (Ph.D.),
is a record of original research carried out

by

AJIBAYE, OLUSOLA

In the Department of Biochemistry

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Olusola

DEDICATION

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ABSTRACT

*Plasmodium falciparum*malaria remains a disease of global andnational health importance due to spread of multi-drug resistance strains of the parasite and insecticide resistance in the mosquito vector resulting in high morbidity and mortality rates. Erythrocyte invasion by *P. falciparum i*s a complex process, of which *P. falciparum* apical membrane antigen I (PfAMA1) and reticulocyte binding protein homolog 5 (PfRH5) are key proteins, hence, targeted for malaria vaccine development. Immune responses against merozoite attachment and invasion may be associated with genetic variations observed in these vaccine candidate antigens. This study assessed polymorphisms in AMA1 and RH5 among *P. falciparum* isolates from Nigeria. One thousand eight hundred and eighty-three (1,883) febrile subjects were examined, out of which 384 (20.39%) were microscopically positive for *P. falciparum* mono-infection. Three hundred of these volunteers were included from three health facilities in Lagos state: Ijede General Hospital, Ikorodu (IJE), Ajeromi General Hospital,Ajegunle (AJE)and St. Kizito Mission Hospital, Lekki, (100 from each site) andEighty age-matched apparently healthy controls from the same communities were included in the study. Giemsa-stained thick and thin blood films of participants were used for *P. falciparum* identification and quantitation. Blood collected on Whatmann 3.0 filter paper was used for DNA extraction and assessed for DNA quality and yield. Molecular genotyping was carried out using the block 3 of Merozoite Surface Protein-2 to determine parasite genetic diversity and Multiplicity of Infection (MOI). One hundred and ninety-five samples which were PCR positive for *msp2* gene were used for PCR amplification and sequencing of *pfama1*and*Pfrh5* genes to determine the genetic structure and polymorphisms on AMA1andRH5. Serum levels of IL-12, TNF-α and IL-1β were determined by Enzyme Linked Immunosorbent Assay (ELISA). Sequence alignment was done usingBioedit and Clustal W in MEGA softwareswhile sequences were analysed using DnaSP. Results revealed that malaria prevalence among the febrile patients was 20.39 %, highest in Ijede and lowest in Lekki. Levels of the pro-inflammatory cytokines studied were higher ($p< 0.05$) in the infected participants than the controls. Eighteen different alleles were observed for MSP-2 loci, FC27 family being more prevalent. Mean MOI was 1.54 ± 0.2. Heterozygosity (H_E) values ranged from 0.77 – 087 and highest for IJE (0.87). Cytokine response was significantly associated with MOI ($P < 0.05$) but not parasite density. Genetic sequence analysis revealed 93 different haplotypes (H) for AMA1 Domain I. Forty-eight of these haplotypes are new with 34 segregating sites, Haplotype diversity of 0.992 ± 0.004, mean nucleotide diversity of 0.02468 ± 0.00076 and Total number of mutations being 52. Tajima's D anddN-dS (non-synonymous minus synonymous mutations) were positive showing positive selection on AMA1. Decline in Linkage Disequilibrium was observed showing high recombination events taking place, however, the High Activity Binding Peptide (HABP) sequences of *rh5* gene revealed three haplotypes of RH5 with negative Tajima's D anddN-dS values showing no selection on RH5. A Single Nucleotide Polymorphism (SNP) (G → A), on nucleotide position 608 was observed on the RH5 sequences based on 3D7 reference sequence. Phylogenetic analyses of the AMA1 and RH5 sequences from this study showed clustering and evidence of evolutionary relationship with 3D7, Guinean AMA1, PAS-2 and FCB-2 RH5 strains using *P. reichenowi* as the out-group at 1000 bootstraps replications. The study indicates that the flow of any resistance alleles among *P. falciparum* isolates in Lagos may be relatively high, with negative impacts on control measures owing to low *fst*values. New AMA1 haplotypes were found suggesting that Nigerian *P. falciparum* should be given consideration in the design and development of control strategies. The study shows that globally efficacious RH5-based

vaccines may be potentially applicable in Nigeria, inspite of the complex genetic diversity of *P. falciparum* isolates from Nigeria.
Key words: Merozoite, polymorphisms, synonymous mutations, haplotypes, heterozygosity.