MOLECULAR BASIS OF POTENTIAL RESISTANCE OF PLASMODIUM FALCIPARUM TO ARTEMISININ BASED COMBINATION THERAPY IN LAGOS AND OSUN STATES, OF NIGERIA

BY

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DECLARATION

We hereby declare that this thesis titles "Molecular basis of potential resistance of*Plasmodium falciparum* to artemisinin based combination therapy in Lagos and Osun States, of Nigeria" is a record of original research work carried out by Tola, Monday in the Department of Cell Biology and Genetics of the University of Lagos, Nigeria.

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CERTIFICATION

This is to certify that the Thesis:

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is a record of original research carried out

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DEDICATION

I dedicate this thesis to my beloved wife and father

Tola OluwatomilayoAdejoke and Tola Patrick

And

To the loving memory of my mother

Tola Omojola Esther

Your life principles has successfully made me the person I am becoming

You will always be remembered

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ABSTRACT

Malaria is a major public health concerndespite more than a century of efforts to eradicate or control it. There are already reports of resistance to almost all antimalarial (including ACT), but there is no such record in Nigeria. This work assessed biochemical and haematological response of malaria infected individuals treated with Artemisinin based Combination Therapy (ACT) and conducted genetic studies to determine molecular basis of resistance of*Plasmodiumfalciparum*to ACT in South West Nigeria.Haematologicalautoanalyzer and chemistry autoanalyzer were used to determine haematological parameters and liver function enzymes activities respectively. Conventional and real time Polymerase Chain Reaction (PCR) assays were used for molecular and expression typing and genes were sequenced using ABI 3730xl genetic analyzer. This study investigated the prevalence of K13-propeller, *pfATPase* 6, *pfmdr* 1 and *pfcrt* gene polymorphisms. Questionnaire which probe into drug use pattern, preference and adverse reaction to ACT was also administered. A total of 135 respondents were interviewed. The respondents had good knowledge of malaria and were of the opinion that, fever (78.6%), vomiting (64.3%), headache (69%) and loss of appetite (83.3%) were the most frequent signs/symptoms of malaria while paleness of the eyes (2.4%) and body weakness (2.4%) were the least mentioned. Of the 135 respondents, 50% use ACT for the treatment of malaria and dosage completion was high as (64.3%) while 60% expressed their willingness to take the drug again due to its effectiveness. The mean PCV were significantly lower ($p>0.05$) in patients with malaria parasite compared to the normal control ranges for both male and female groups. The mean platelet values decreased significantly ($p<0.05$) before treatment with no difference observed after treatment compared to control but Neutrophil values observed for the days of study were significantly ($p<0.05$)decreased compared to control. The WBC and lymphocyte had mean values that were not significantly different ($p>0.05$) from the control. Malaria positive patients had a significantly ($p<0.05$) higher mean activity values of the various liver function enzymes (Aspartate aminotransferase, Alanine aminotransferase and Alkaline phosphatase) compared to control mean values. From multiplex PCR method was carried out for species identification,the results showed a total *Plasmodium falciparum* parasite in the studied population. Different expression patterns of target genes (K13 and *pfATPase*) were observed in malaria parasite transcript. The wild strain of K13 gene was found in the parasite population while *pfATPase* 6 had very low expression generally; both the wild and mutant strains were expressed. The analysis of single nucleotide polymorphisms (SNPs) in drug resistance associated parasite genes is a potential alternative to classical time- and resource-consuming *in vivo* studies to monitor drug resistance. Eight (8) different mutations were detected in K13 gene (G497S, R539F, I543V, A557?, V566K, A578K, C580Y, and D584I) with A557 having the highest prevalence in the parasite study population. One (1) synonymous (S679S) and two (2) non-synonymous (M699V, S769M) mutations were detected in the *pfATPase* 6 gene. Two non-synonymous (N86K and Y184F) mutations were detected in *pfmdr* 1 while *pfcrt* haplotype 72-76 mutation for antimalarial drug resistance common in Africa (CVIET) had a prevalence of 45% in the parasite study population. Point mutations on K13, *pfATPase* 6 and other genes that are associated with ACT resistance indicating imminent ACT resistant parasite were observed.

Key words: Malaria, Antioxidant enzyme, Haematology, Drug resistance, Artemisinin