PARASITOLOGIC AND MOLECULAR ASSESSMENT OF SOME ANTIMALARIAL DRUGS USED IN ASYMPTOMATIC MALARIA IN PREGNANCY IN LAGOS

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

BY

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MAY 2011

DECLARATION

PARASITOLOGIC AND MOLECULAR ASSESSMENT OF SOME ANTIMALARIAL DRUGS USED IN ASYMPTOMATIC MALARIA IN PREGNANCY IN LAGOS

BY

AGOMO, CHIMERE OBIORA

THIS IS TO CERTIFY THAT THIS RESEARCH WORK WAS CARRIED OUT BY

AGOMO, CHIMERE OBIORA

UNDER OUR SUPERVISION

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CERTIFICATION

This is to certify that the thesis:

"Parasitologic and Molecular Assessment of Some Antimalarial Drugs Used in Asymptomatic Malaria in Pregnancy in Lagos"

Submitted to the School 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

AGOMO, CHIMERE OBIORA
In the Department of Medical Microbiology and Parasitology.

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DEDICATION

To my wife, children and Almighty God

ACKNOWLEDGEMENTS

The Tropical Diseases Research Laboratory of the Department of Medical Microbiology and Parasitology, University of Lagos provided the platform for re-training in malaria microscopy and influenced the choice of hospitals used. I thank Dr Wellington Oyibo for the invaluable mentorship he provided day and night. My profound gratitude goes to Prof. A.F. Fagbenro-Beyioku for her supervision and critical evaluations.

I thank Dr P.U Agomo, my uncle and a Director of Research in Nigerian Institute of Medical Research, Lagos for his financial and moral support. The encouragement and challenge to obtain a PhD came from him. The field studies were made possible by the wonderful assistance from Mr Onimisi I Raji, Mrs Adepeju, Mr Innocent Akpan and Mrs Linda Ubeh for their roles in questionnaire administration and follow-up of study participants.

My sincere gratitude goes to management and staff of St. Kizito Primary Healthcare center Lekki and Ajeromi General Hospital Ajegunle for their cooperation and granting access to the pregnant women attending their antenatal clinics. Special thanks to Drs Chiara Mezillara, Funke Odukoya-Maije , Ehile and Rose Anorlu for their invaluable support during the conduct of the field studies.

The drugs (sulphadoxine-pyrimethamine) were kindly donated by IPCA Pharmaceuticals PLC and Emzor Pharmaceuticals PLC.

I am grateful to Dr Carly Roper and Dr Colin Sutherland of the Department of Immunology, London School of Hygiene and Tropical Medicine, London for the laboratory space, reagents and technical assistance with the molecular studies, Dr Sutherland and his team (Drs Rachael Hallet, Mary Oguike, Sabelo Dlamini, Nahla Gadalla, Khalid Beshir) were wonderful, always ready to proffer solutions on and off the laboratory bench.

I am thankful to the Malaria Research Unit of the Department of Biochemistry, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos for their support throughout the program. I appreciate the moral and technical support of all my friends and colleagues such as: Drs OO Aina, Olukosi YA, Oladosu Oladipo, Mr Paul A Obi and Charles Ezeakku.

I am grateful to my father and late mother for the sound educational foundation they laid for me. To my loving wife Amarachukwu, and my children, Chukwuemeka, Nneoma, Ebube and Onyinye, I say thank you for standing by me during the difficult times. I sincerely appreciate the love and support of my sisters, brothers, nephews and nieces.

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Abstract

The prevention of malaria in pregnancy (MIP) reduces the adverse effect of malaria on both mother and foetus, however the level of MIP in Nigeria is not clear considering high variation in published reports. The prospects of intermittent preventive treatment of malaria during pregnancy with sulphadoxine-pyrimethamine (IPTp-SP), a recommended malaria control strategy during pregnancy, lie in the therapeutic efficacy of sulphadoxine-pyrimethamine (SP) and high compliance to the dosing regimen. Reports of increasing level of resistance to SP in non-pregnant populations have however necessitated continuous monitoring of the efficacy of SP in pregnant women. Despite the adoption of IPTp-SP, the use of chloroquine and pyrimethamine for antimalaria chemoprophylaxis is still common. Molecular typing of resistance markers is a useful tool for surveillance of antimalarial drug resistance. The objectives of this research were to determine the prevalence of malaria and anaemia in pregnancy in Lagos; determine the protective efficacy of IPTp-SP and the **equivalence of** monthly IPTp-SP to two-dose IPTp-SP; and to describe *Pfcrt*, *pfmdr1,* and *dhfr* genes of *P. falciparum* isolates from pregnant women in Lagos. Pregnant women attending antenatal clinics of Ajeromi General Hospital, Ajegunle and St. Kizito Primary Health Center, Lekki between May 2007 and February 2008 were recruited. Demographic characteristics and malaria prevention practices of the pregnant women were captured with an interviewer-administered semi-structured questionnaire. Malaria parasitaemia, packed cell volume and total leukocyte counts were determined by standard methods. For the equivalence study, malaria parasitaemia was monitored monthly and pregnancy outcome assessed. Haplotypes of *Pfcrt* were assayed using real-time PCR, while *Pfmdr1 and dhfr* genes were sequenced to determine point mutations on the genes. The prevalence of malaria and anaemia among the 1084 pregnant women recruited into the study were 7.7% and 52.3% respectively. *Plasmodium falciparum* was the major species found (95.2%). Anaemia was significantly associated ($P<0.01$) with malaria infection and

gestation age but not with gravidity of the women. Significant reduction in malaria infection (P<0.05) was associated with the use of insecticide sprays (RR = 2.79, 95 C.I. = 1.84-4.22); and the combined use of insecticide spray and insecticide-treated nets (ITN) (RR = 6.53, 95% C.I. 0.92-46.33). Factors identified to increase the risk of malaria infection include young maternal age (<20years), gravidity (primigravidae) and occupation. In phase two, 122 women were recruited into the two-dose arm (Arm A) while 137 were recruited into the monthly dose arm (Arm B). A total of 8 (3.1%) had parasitaemia at recruitment (M0): 5(4.1%) and 3(2.2%) in Arms A and B respectively. The overall protective efficacy of IPTp-SP was 98.4% (Arm A 98.3% and Arm B 98.5%) at Month 1 (P=0.636). A woman became parasitaemic at Month 2 in Arm A but none in Arm B after Month 1. The outcome of pregnancy (low birth weight and live births) was similar within the two study arms (P>0.05) irrespective of gravidity and age of the women. The frequency of *Pfcrt* haplotypes were: CVIET 29(53.7%), CVMNK 13(24.1%), CVIET+CVMNK 12(22.2%); *pfmdr1* haplotypes: NYSND 15(53.6%), YYSND 5(17.9%), NFSND 6(21.4%), YFSND 2(7.1%); dhfr haplotypes: ACNCSVI 4(26.7%), ACICNVI 1(6.7%) ACIRNVI 10(66.7%). Point mutations in *Pfmdr1* were 86Y 7(25.0%), 184F 8(28.6%). No mutation was observed at codons 1034, 1042 and 1246. The *pfmdr1 86N* and *184Y* were the predominant haplotypes irrespective of gravidity of the women. However, there was no significant association (P>0.05) between the *Pfcrt* haplotypes and *Pfmdr1* mutations and gravidity of the pregnant women from which the parasites were isolated. The *Pfmdr1* 86Y was significantly associated with antimalarial chemoprophylaxis with chloroquine (P<0.05). In conclusion, this study exposes the over-diagnosis of MIP and the need to employ quality assurance procedures. IPTp-SP was found to be effective; and 2-dose IPTp-SP is equivalent to monthly IPTp-SP in Lagos. The high levels of resistance markers for pyrimethamine and chloroquine recorded in this study suggests that treatment or chemoprophylaxis of falciparum malaria in pregnancy with any of these two drugs may not be effective.