

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

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BY

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CERTIFICATION

This is to certify that the thesis:

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DEDICATION

To my wife, children and Almighty God

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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 *Pfcr*, *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 *Pfcr* 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 *Pfcr*t haplotypes were: CVIET 29(53.7%), CVMNK 13(24.1%), CVIET+CVMNK 12(22.2%); *pfmdr*1 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 *Pfmdr*1 were 86Y 7(25.0%), 184F 8(28.6%). No mutation was observed at codons 1034, 1042 and 1246. The *pfmdr*1 86N and 184Y were the predominant haplotypes irrespective of gravidity of the women. However, there was no significant association ($P > 0.05$) between the *Pfcr*t haplotypes and *Pfmdr*1 mutations and gravidity of the pregnant women from which the parasites were isolated. The *Pfmdr*1 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.