Malaria diagnosis, Prevalence of drug resistance in *Plasmodium falciparum* and gene flow patterns in endemic and epidemic regions of Kenya

Laura Nyawira Wangai

A thesis submitted in partial fulfillment for the degree of Doctor of philosophy in molecular medicine in the Jomo Kenyatta University of Agriculture and Technology

2009

ABSTRACT

Plasmodium falciparum strains resistant to the commonly used drugs are becoming increasingly widespread in Kenya. The emergence and spread of resistant strains is an impediment to efforts to manage malaria. The spread of drug resistance is due to spread of drug resistance genes. Resistance to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) has been shown to lower the efficacy of these drugs. Resistance to artemisinin-based combination therapy (ACT) has also been shown in some *in vitro* studies. Therefore, as artemisinin-based combination therapy (ACT) is becoming widely used in sub-Saharan Africa, there is need for regular and comprehensive surveillance of resistance to this therapy. The study aimed at Malaria diagnosis, determining the prevalence of SP, CQ and artemisinin resistance gene markers and gene flow patterns. A total of 512 samples were analysed in this study. Among these, 442 samples were collected from Mbita an endemic area and 8 epidemic areas during the year 2007. In addition 36 samples collected in 1998 from Oyugis an endemic area and 24 samples collected in 2007 from Brazil-America were also included in the study. Conventional and nested PCR were performed and the products analysed by gels, fragment analysis and sequencing. The Gene mapper, Seqscape and Genepop softwares were used for sequence and geneflow analysis. In epidemic areas PCR picked 72 (20.6%) out of 350 samples that had initially been found to be negative by microscopy as positive for *Plasmodium falciparum*. Prevalence of chloroquine resistance markers was found to have declined significantly between 1997 and 2007 (Chi test, p=0.0190, p=0.0159 for *pfmdr1* and *pfcrt* respectively). This study showed that when hybridized with the MEK(combined probe for *pfcrt*), only 3 samples (7.9%) from epidemic zones, 4 samples (11.42%) from Oyugis and 7 samples (10.9%) from Mbita hybridized. 11 (28.94%), 22 (62.86%) and 32 (50.00%) samples from epidemic sites, Oyugis and Mbita respectively hybridized to the MET probe. One (2%)

sample from sites, 8 (22.36%) samples from Oyugis and 21(32.81%) samples from Mbita hybridized to both MEK and MET. When *pfmdr1*-86 was analyzed by dot blot, 8(21.05%), Two (5.71%) and 18(32.14%) samples from epidemic sites, Oyugis and Mbita respectively had wild type allele N86, 24 (63.15%), 19 (52.70%) and 37 (57.81%) samples from epidemic sites, Oyugis and Mbita respectively had mutant allele Y86.At the introduction of SP as the first line drugs, prevalence of the mutant isolates was significantly low (p= 0.0001) compared to 10 years later in the endemic and epidemic areas. The mutants increased from 16.7% in 1998 to 78.9% in the year 2007. The study detected neither S769N nor A623E *pfatpase*6 mutations in 30 of the samples from Mbita sequenced. There was no mutant *pfatpase6* codon detected after sequencing. Gene flow occurred between and within endemic and epidemic regions having no significant population differantion (p>0.05). However there was a highly significant population differentiation (p< 0.0001) between Brazil and Kenyan sites. This study has demonstrated that anti-malarial drugs resistance is influenced by respective drug pressure, misdiagnosis and the spread of the mutant genes within the population. This study is important in ensuring proper treatment based on proper diagnosis and use of effective drugs.