ABSTRACT

Recent decreases in the global malaria burden are partly due to the deployment of artemisinin-based combination therapies (ACTs) for treatment of uncomplicated *Plasmodium falciparum* malaria. However, these significant gains are threatened by emergence of drug-resistant malaria parasites. In Africa, *P. falciparum* remains susceptible to ACTs. However, due to the emergence of parasites that are resistant to ACTs in Southeast Asia and the recent report of declined responsiveness of *P. falciparum* infections to ACTs at the Kenyan coast, there is need for continuous monitoring of ACT resistance in high malaria transmission areas such as western Kenya. Currently, therapeutic efficacy studies (TES) are considered the gold standard for determining antimalarial drug efficacy. However, the World Health Organization recommends that TES be complemented with surveillance for molecular markers associated with parasite resistance to monitor the emergence of resistance before it leads to widespread clinical resistance. Therefore, surveillance for mutations in *P. falciparum kelch 13* (*Pfk13*) propeller domain and *P. falciparum multi-drug resistance protein 1* (*Pfmdr1*) gene, which have been implicated in parasite resistance to ACTs, is critical in informing the current status of resistance to ACTs. This study characterized the mutation profile of *Pfk13* propeller region and *Pfmdr1* gene in a malaria endemic area of western Kenya. The specific objectives were to determine; the mutations in *Pfk13* propeller region (codons 458, 493, 539, 543, 561 and 580); the mutations in *Pfmdr1* gene (codons 86, 184, 1034, 1042 and 1246) and association with treatment outcome. This laboratory based experimental study used archived blood samples from a recently completed TES (2016-2017) which assessed the efficacy of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) in children aged 6-59 months in western Kenya. A total of 423 samples which included 323 samples collected pre-treatment (day 0) and 110 samples collected on the day of recurrent parasitaemia (up to day 42) were analyzed. Parasite genomic DNA was extracted from dried blood spots using QIAamp DNA Mini Kit as described by the manufacturer. Single nucleotide polymorphisms was determined by nested Polymerase Chain Reaction and Sanger sequencing. Sequencing of the *Pfk13* gene was successful for 93.8% samples. For all the samples tested, none of the *Pfk13* mutations that have been associated with artemisinin resistance was detected. However, other non-synonymous mutations which have not been associated with resistance were detected.; for example; Out of 317 day 0 samples, 2 (0.6%) had S522**C**, 5 (1.6%) had A578**S,** 1 (0.3%) E596**D** mutations and 309 (97.5%) were wild type. Out of 95 recurrent infection samples, 2 (2.3%) had A578**S,** 1 (1.1%) had C580**F** and 85 (96.6%) samples were wild type. For multidrug resistant marker *Pfmdr1*, 95.8% samples were successfully sequenced. Out of 320 day 0 samples, 1 (0.3%) had N86**Y**, 192 (59.7%) had Y184**F**, 30 (9.4%) had D1246**Y** mutations and 117 (36.6%) samples were wild type (NYSND). Out of 95 recurrent infection samples, 1 (1.1%) had N86**Y**, 59 (62.1%) had Y184**F**, 5 (5.3%) had D1246**Y** mutations and 33 (34.7%) samples were wild type (NYSND). There was no statistically significant association between any of the observed *Pfk13* mutations with treatment outcome for both AL treatment arms (S522**C** and C580**F**; Fisher statistic; $p = 1$ and $p = 0.16$ respectively) and DP treatment arm (S522**C** and A578**S**; Fisher statistic; $p = 1$ and $p = 0.22$ respectively). Although a high frequency of *Pfmdr1* Y184**F** mutations was detected, there was no statistically significant association between these mutations with treatment failure (recrudescence) in both treatment arms (Fisher statistic; $p = 1$ and $p = 0.735$, for AL and DP treatment arms respectively). These results indicate absence of mutations associated with parasite resistance to ACTs in western Kenya. However, continued monitoring for molecular v

markers of ACT resistance is needed for providing timely evidence-based malaria treatment policies in western Kenya and other malaria endemic regions.