

Full Length Research Paper

Selection of *Plasmodium falciparum* *Pfmdr-1* N86Y alleles by Amodiaquine-Artesunate and Artemether-Lumefantrine in Nanoro, Burkina Faso

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Plasmodium falciparum *Pfmdr1-86* gene polymorphisms were investigated in blood samples of patients > 6 months of age treated with Amodiaquine-Artesunate (ASAQ) and Artemether-Lumefantrine (AL) in Nanoro, Burkina Faso. Treatments outcome was determined with a 28-day follow-up. The prevalence of *Pfmdr-1* N86Y alleles was determined before and after treatment. The PCR-adjusted Adequate Clinical and Parasitological Response (ACPR) was higher in the ASAQ arm (100%) than in the AL arm (87.5%) [Risk difference = -12.50; 95% CI: -20.13; - 4.86 (p=0.001)]. The prevalence of *Pfmdr-1* Y86 mutation in the ASAQ arm was significantly higher among patients who had a recurrent parasitaemia (54.54%) than those classified as ACPR (12.70%) (p = 0.007). Similarly, the prevalence of the mutant allele *Pfmdr-1* Y86 before treatment (20.00%) was significantly lower than that found in post-treatment (55.56%) in the ASAQ arm (p = 0.01). However, we did not see such difference in the AL arm either for post-treatment samples versus pre-treatment samples (p = 0.88), nor for patients with recurrent parasitaemia compared to those classified as ACPR (p = 0.65). In conclusion, our study showed that ASAQ is selected for parasites carrying the *Pfmdr-1* Y86 mutation; however we were not able to demonstrate the reverse relationship between *Pfmdr-1* 86N and AL treatment as previously reported in Africa.

Key words: Malaria, *Plasmodium falciparum*, Amodiaquine-Artesunate, Artemether-Lumefantrine, Efficacy, *Pfmdr-1*, Burkina Faso.

INTRODUCTION

Worldwide use of Artemisinin-based combination therapies (ACT) combined with other control measures (vector control, seasonal malaria chemoprophylaxis,

intermittent preventive treatment in pregnant women, etc) has led to significant decreases in malaria transmission and thus subsequent malaria-related morbidity and

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mortality over the past decade in many endemic countries (Maude et al., 2009; WHO, 2006). Nevertheless, malaria still kills approximately 584,000 people a year worldwide and causes illness in hundreds of millions more, most of them children living in sub-Saharan Africa (World Malaria report 2013).

ACTs, mostly artemether–lumefantrine (AL) and artesunate–amodiaquine (ASAQ), are deployed worldwide, including Burkina Faso where a new treatment policy was adopted in 2005 recommending the use of ASAQ or alternatively AL for the treatment of uncomplicated falciparum malaria (Gansané et al., 2009; Zwang et al., 2009). However, following the reports on the decreasing susceptibility of *P. falciparum* to artemisinin derivatives along the Thailand and Myanmar border (Dondorp et al., 2009; Lim et al., 2009; Noedl et al., 2010; Rogers et al., 2009), several studies have reported a decline of adequate parasitological response rate to treatment with ACTs in African patients, possibly due to the emergence of parasites with reduced drug sensitivity (Beshir et al., 2013; Borrmann et al., 2011). In such context, the regular monitoring of *P. falciparum* sensitivity against artemisinin derivatives and its partner drugs in Africa is needed. Several studies have identified the polymorphisms of *Pfmdr-1* N86Y gene as one of the main molecular markers involved in the development of tolerance / resistance to Lumefantrine and Amodiaquine; the two partner drug of AL and ASAQ respectively (Baliraine and Rosenthal, 2011; Baraka et al., 2015; Dokomajilar et al., 2006; Eyase et al., 2013; Holmgren et al., 2006, 2007; Sisowath et al., 2005, 2007; Somé et al., 2010). Here we report the results of a pilot study investigating the relationship between the polymorphism of the *Pfmdr-1* N86Y alleles and the treatment outcome of ASAQ and AL in Burkina Faso.

MATERIAL AND METHODS

The study was carried out between October and December 2012 in Nanoro, Burkina Faso where malaria is hyper-endemic with a seasonal transmission from July to December. *Plasmodium falciparum* is the predominant malaria parasite and the commonest vectors are *Anopheles gambiae* ss, *An. funestus* and *An. Arabiensis* (Tinto et al., 2002). This was part of a pilot study that investigated the therapeutic efficacy of AL and ASAQ in patients \geq 6 months of age with a molecular markers study nested into it. The study methodology has been described in detail elsewhere (ClinicalTrials.gov Identifier: NCT01697787). Briefly, patients with fever (axillary temperature of 37.5°C) or history of fever with a suspicion of malaria were screened after informed consent. Then, patients meeting the inclusion criteria were treated and followed up according to the WHO 28-day *in vivo* test (WHO, 2003). Outcomes were defined according to the WHO guidelines for monitoring antimalarial drug resistance (WHO, 2003).

Blood samples for the molecular analysis were collected on filter paper (Whatman 3, Maidstone, England) at day 0 before treatment and at the time of recurrent parasitaemia. DNA was extracted from dried blood spots using Qlamp DNA miniKit (Qiagen, Germany) following the manufacturers procedures. Detection of *Pfmdr1* N86Y polymorphisms was performed using nested PCR method followed by a restriction fragment length polymorphism (RFLP) (Dokomajilar

et al., 2006): Briefly, the first round was done by using primers MDR1 5'-ATGGGTAAAGAGCAGAAAGA-3' and MDR2 5'-AACGCAAGTAATACATAAAGTCA-3' and then nested PCR was done by using primers MDR3 5'-TGGTAACCTCAGTATCAAAGAA-3' and MDR4 5'-A TAAACCTAAAAAGGAACTGG-3'. The second round (nested PCR) product was subjected to enzyme digestion with *AfIII* (New England Biolabs), which cuts only the mutant gene into 226 bp and 295 bp fragments. For each series of samples, water was used as a negative control, 3D7-clone DNA was used as the wild-type control and Dd2- DNA was used as the mutant control. Nested PCR was performed as well for the analysis of *Msp-1* and *Msp-2* to distinguish between recrudescence and new infection (Ranford-Cartwright et al., 1997). Data were double entered in an Excel database. Statistical analysis was performed using STATA (IC), version 8.0 software. *Pfmdr-1* N86Y genotype was determined by the presence or absence of wild/mutant alleles. Differences between groups were assessed using the Chi-square test for proportions and a P-value \leq 0.05 was considered as statistically significant.

RESULTS

Out of 246 patients screened, 78.45% (193 patients) had a microscopically confirmed malaria infection. Out of them, 150 were randomized to receive either ASAQ (n=75) or AL (n=75). The two treatments' outcomes are summarized in Table 1. At day 28, 74 patients completed their follow-up in the ASAQ arm against 72 patients in the AL arm. Unadjusted Adequate Clinical and Parasitological Response (ACPR) at day 28 was significantly higher in the ASAQ (85.13%) than in the AL arm (61.11%) [Risk difference = -24.13; 95% CI: -38.00; - 10.25 (p=0.001)]. Similarly, The PCR-adjusted ACPR was significantly higher in the ASAQ (100%) than in the AL arm (87.5%) [Risk difference = -12.50; 95% CI: -20.13; - 4.86 (p=0.001)]. The *Pfmdr-1* N86Y gene was successfully genotyped in the blood samples of the 150 patients randomized at day 0. The overall prevalence of the mutant allele *Pfmdr-1* Y86 was 18.67% [CI 95% (12.78 to 25.84)].

The prevalence of the two alleles before treatment in relation with treatments outcomes is summarized in Table 2. The prevalence of *Pfmdr-1* Y86 mutation in the ASAQ arm was significantly higher among patients who had a recurrent parasitaemia (54.54%) than those classified as ACPR (12.70%) (p = 0.007). However, we did not see such difference in the AL arm (p = 0.65). Similarly, the prevalence of the mutant allele *Pfmdr-1* Y86 before treatment (20.00%) was significantly lower than that found in post-treatment (55.56%) in the ASAQ arm (p = 0.01). However, we did not see such difference in the AL arm (p = 0.88).

DISCUSSION

Good efficacy of an ACT is reported when the partner drug is also efficacious (The Four Artemisinin-Based Combinations (4ABC) Study Group, 2011). The high rate of 100% ACPR reported in the ASAQ arm is an indication

Table 1. Efficacy rates of ASAQ and AL at day 28

Outcome	AL (n=72)	ASAQ (n=74)	Difference [95%CI]	P-value
PCR unadjusted				
ACPR	44 (61.11)	63 (85.13)		
ETF	0 (0.0)	0 (0.0)		
LCF	15 (20.83)	4 (5.41)	-24.13 [-38.00 ; -10.25]	0.001
LPF	13 (18.06)	7 (9.46)		
TTF	28 (38.89)	11 (14.87)		
PCR adjusted*				
ACPR	63 (87.5)	74 (100)		
ETF	0 (0.0)	0 (0.0)		
LCF	0 (0.0)	0 (0.0)	-12.5 [-20.13 ; -4.86]	0.001
LPF	9 (12.5)	0 (0.0)		
TTF	9 (12.5)	0 (0.0)		

AL: Artemether-Lumefantrine; ASAQ: Artesunate-Amodiaquine; ACPR: Adequate Clinical and Parasitological Response; ETF: Early Treatment Failure; LCF: Late Clinical Failure; LPF: Late Parasitological Failure; TTF: Total treatment Failure.

Table 2. Selection of *Pfmdr-1* N86Y alleles by ASAQ and AL.

Treatment outcome	ASAQ		AL	
	Wild(N86)	Mutant(Y86)	Wild(N86)	Mutant(Y86)
TTF % (n/N)	0(0/0)	0(0/0)	66.66 (6/9)	33.34 (3/9)
New infection % (n/N)	45.46 (5/11)	54.54(6/11)	94.73 (18/19)	5.27 (1/19)
ACPR% (n/N)	87.30 (55/63)	12.7 (8/63)	81.81 (36/44)	18.18 (8/44)

AL: Artemether-Lumefantrine; ASAQ: Artesunate-Amodiaquine; TTF: Total Treatment Failures; ACPR: Adequate Clinical and Parasitological Response.

of a good efficacy of Amodiaquine and confirm the results of previous studies conducted in Burkina Faso and comparing the two ACTs (Siribié, Diarra, Tiono, Soulama, & Sirima, 2012; Sirima et al., 2009; The Four Artemisinin-Based Combinations (4ABC) Study Group, 2011; Tinto et al., 2008, 2014; Zongo et al., 2007). The overall prevalence of the mutant allele *Pfmdr-1* Y86 regardless of the treatment arm was surprisingly low when compared with that reported by previous studies; indicating a decrease of this mutation in Burkina Faso (Baraka et al., 2015; Somé et al., 2010; Tinto et al., 2003, 2008).

The *Pfmdr-1* Y86 and *Pfcr1* T76 mutations have been identified as the main determinants for 4-aminoquinolines resistance including Amodiaquine (Tinto et al. 2003; Djimé et al. 2015; Sondo et al. 2015). The decrease of the *Pfmdr-1* Y86 mutation observed in our study may follow the same trend observed with *Pfcr1* T76 mutation after the malaria treatment policy change in endemic countries. Indeed, a decrease of the prevalence of *Pfcr1* T76 mutation was reported following the withdrawal of chloroquine from the treatment policy in many Africa endemic countries (Kublin et al., 2003; Laufer et al.,

2010; Mwai et al., 2009; Sondo et al., 2015). These findings suggest that Amodiaquine resistance may be decreasing following the implementation of the new anti-malarial drug policy based on ACT in Burkina Faso.

An association between the polymorphism in *Pfmdr-1* gene and the parasite response to arylaminoalcohols including lumefantrine has been reported with a significant increase of *Pfmdr-1* 86N wild type allele after exposure to the drug; suggesting that Lumefantrine exerts the opposite effect of amodiaquine on this locus (Duraisingh and Cowman, 2005; Sisowath et al., 2005). In addition, *Pfmdr1* 86N-carrying parasites have been associated with decreased sensitivity to lumefantrine *in vitro*, suggesting this allele as a potential marker of lumefantrine resistance (Baliraine and Rosenthal, 2011). We noticed in our study an overall high prevalence of *Pfmdr-1* 86N allele. However we did not observed an increase in the prevalence of the *Pfmdr-1* 86N in patients who had a recurrent parasitaemia samples than those classified as ACPR. Similarly, we did not observe an increase of the prevalence in the post-treatment samples than in pre-treatment samples. Therefore there was no significant selection of the *Pfmdr1* 86N allele after AL

treatment in our study as reported previously in Africa (Baliraine and Rosenthal, 2011; Sisowath et al., 2005, 2007).

Overall, we did not see any linear association between the *Pfmdr1*-N86Y alleles and both AL and ASAQ treatments outcome. This lack of relationship confirms the difficulty to predict the individual treatment outcome by looking at molecular markers alleles in pre-treatment samples (Holmgren et al., 2006). This can be explained by the effect of the host immunity which could modify the relation between molecular markers and resistance; a phenomenon similar to what has been reported previously for CQ resistance (Djimé et al., 2015; Tinto et al., 2003, 2008). Indeed, in our study, patients of all age groups including adults were enrolled when in most of studies where this relationship was established, study participants were mostly children (Baliraine and Rosenthal, 2011; Sisowath et al., 2005, 2007). However, further studies should be carried out exclusively in children to confirm this assumption in our study area.

In conclusion, our study showed a decrease of the *Pfmdr1* Y86 mutation in parasites strains circulating in Burkina Faso. ASAQ selected for parasites carrying the *Pfmdr1* Y86 mutation, however we were not able to demonstrate the reverse relationship between *Pfmdr1* 86N allele and AL treatment as reported previously.

Conflict of interests

The authors have not declared any conflict of interests.

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