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Rapid increase in resistance of *Plasmodium falciparum* to chloroquine-Fansidar in Uganda and the potential of amodiaquine-Fansidar as a better alternative

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Abstract

Combinations of chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) [CQSP] as the first line agents in Uganda have replaced CQ monotherapy. The idea of the combination is to delay the development of malaria resistance to either drug when used alone. We compared the clinical, parasitological and molecular findings of two studies with treatment arms of CQSP, amodiaquine (AQ) plus SP (AQSP) both done in 2003 with a study done 1 year earlier (2002) using SP alone. There was a notable decrease in adequate clinical response (ACR) by day 14 from 92.7% with SP to 80% with the combination CQSP, a year later. AQSP combination was found to have the best effect (94.3% ACR). There were no early treatment failures in the AQSP group. However, treatment failures were recorded at 20% on day 14 and 43% on day 28 for CQSP treatment and 5.7% by day 14 and 28.8% by day 28 in the AQSP group. The number of mutations that are associated with SP resistance increased from 2002 to 2003 at all loci monitored, from 83.8 to 100% at codon 108, 58.7 to 76% at codon 59 in the DHFR gene, and from 58.8 to 86% at codon 437 and 33 to 43% at codon 540 in the DHPS gene. We conclude that there has been a rapid development of resistance since the introduction of the new policy guidelines. AQSP was found to be a superior drug combination compared to CQSP and could be used as a low cost alternative at the moment.

Keywords: Resistance; *Falciparum*; Fansidar; Chloroquine; Amodiaquine

1. Introduction

Malaria remains one of the most serious global health problems and a leading cause of childhood morbidity and mortality, especially in Africa. Efforts to control malaria in Africa have been severely compromised by the emergence of resistance in *Plasmodium falciparum* to the inexpensive and widely used drugs, chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) (Attaran et al., 2004). In Uganda, chloroquine resistance levels approached a national average of 40% (Kamya et al., 2001, 2002) by the year 2000 and as

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a consequence, even with limited data, the Ugandan Ministry of Health recommended a combination of CQ and SP (Ugandan Ministry of Health, 2000a,b) as an interim policy for the following reasons. Firstly, chloroquine is cheap, available, tolerable and has antipyretic properties regardless of its effect on the malaria parasites (Barat et al., 1998; Brandling-Bennett et al., 1988). Secondly, the long-term effectiveness of SP was debatable since the countries that were using it as first line treatment had began to register high levels of resistance (Nevill and Gardner, 1991; Nwanyanwu et al., 1996; Omar et al., 2001; Verhoeff et al., 1997). Thirdly, while there was only limited data on the other available antimalarial agents in Uganda, earlier studies in Asia and Tanzania (McIntosh, 2001) had showed high efficacy levels with the CQSP combination.

Recently, a number of separate studies have been done to establish the efficacies of drug combinations. In a comparative study done in Kampala, CQ/SP appeared preferable to SP alone in general, but when results were stratified by age, the benefit was only significant in patients below 5 years of age (Gasasira et al., 2003). The AQ/SP combination was much more efficacious and this confirmed earlier studies that had shown AQ to be a better treatment than CQ or SP (Gasasira et al., 2003; Staedke et al., 2001). Another study done in Tororo, Uganda, found AQ/SP 100% efficacious as compared to 93% for CQ/SP and 91% for SP alone. Parasitological failure by day 28, however, occurred in 16, 48 and 61% of the patients in the three treatment arms (AQ/SP, CQ/SP, SP), respectively (Talisuna et al., 2004a). It was found that addition of CQ to SP did not offer any added therapeutic advantage (Talisuna et al., 2004a; Checchi et al., 2004), but whether it would delay the development of resistance to the component drug was not addressed. It is also notable that, since CQ plus SP replaced CQ alone as the first-line antimalarial drug, only few reports have been published from continuous evaluations of clinical, parasitological and molecular profiles of *P. falciparum* resistance patterns.

Genetic variation associated with both CQ and SP resistance can be monitored with specific molecular markers. Position 76 in the *Pfcrt* gene which is putatively associated with *P. falciparum* resistance to CQ was found to be 100% mutated in Kampala (Dorsey et al., 2001; Kyosiimire-Lugemwa et al., 2002) and we recently confirmed these findings by similar analysis of over 200 isolates from patients at Kasangati Health Centre (results not shown). Therefore, in the present study, we did not study the *Pfcrt* gene. *P. falciparum* resistance to SP has been reported to be associated with point mutations in genes that encode two key enzymes, dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), which are the targets for pyrimethamine and sulfadoxine, respectively (Wang et al., 1997). The accumulation of mutations in DHFR (108-Asn, 51-Ile, 59-Arg) and DHPS (437-Gly, 540-Glu) appears be valuable in detecting emerging resistance before clinical treatment failure is evident (Hastings et al., 2002; Kyabayinze et al., 2003). Some studies found mutations at codons 108 and 51 too frequent to be practical predictors of resistance and only three mutations (DHFR Arg 59, DHPS 437 and 540) were identified as helpful in correlating with clinical outcome (Kyabayinze et al., 2003; Staedke et al., 2004; Talisuna et al., 2003). Another study has revealed that mutations at DHFR codons 108 and 59 together with DHPS 540 are related to parasitological failure (Talisuna et al., 2004b). Based on the above recent findings, we examined four mutations at DHFR 108 and 59 and at DHPS 437 and 540 as the markers to monitor the rate of development of *P. falciparum* resistance to SP. Most clinical studies of malaria drug resistance, which apply the WHO guidelines of assessing clinical or parasitological outcome (WHO, 2001), are limited as they do not distinguish recrudescence of resistant parasites from re-infection by new parasite strains. For this, a simple genotyping system using only merozoite protein 2 gene (MSP 2) to type infecting parasites adequately was described recently (Cattamanchi et al., 2003).

In this report, subsequent to the recent policy change from CQ alone to CQ plus SP as first-line therapy for uncomplicated malaria in Uganda, we compared the clinical efficacies of CQSP and AQSP combination therapies with SP alone while we monitored parasitological outcomes and development of gene mutations associated with *P. falciparum* SP resistance.

2. Materials and methods

2.1. Subject recruitment

Studies were conducted at Kasangati Health Centre, an outreach health unit for Makerere University