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Case Report of Attenuated-Responsiveness to Coartem[®] in Western Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. This work was carried out in collaboration between all authors. Author EK had protocol oversight, wrote first draft of manuscript, conception and design of manuscript and provided initial data analysis and interpretation. Author HMA designed protocol, designed laboratory procedures, contributed to the writing of first manuscript draft, data analysis and interpretation. Author AOA carried out molecular assays, was involved in drafting the manuscript, revising it critically for important intellectual content. Author RY carried out in vitro assays and data analysis, also took part in reviewing of the manuscript. Author LAI carried out molecular analyses, data analysis, interpretation and manuscript revision. Author ACC designed and implemented in vitro drug testing, data analysis and interpretation, revising it critically for important intellectual content. Author DWJ carried out molecular analyses, data analysis and managed the literature searches. Author CO carried out drug testing assays and preliminary pharmacokinetics analyses. Author DO was instrumental in manuscript revision and design and implementation of protocol. Author CM identified/detected case at point of care, undertook acquisition initial processing of clinical and laboratory data. Author EAO identified/detected case at point of care, designed and implemented of the protocol, case management and acquisition of clinical data. Author PO performed the point of care case confirmation and management, clinical data analysis and interpretation. Author AO confirmed case, took part in clinical data analysis and interpretation, protocol design. Author BA designed the protocol, participated in the manuscript conception, design and writing, data analysis and interpretation. Author BO designed and implemented the protocol, analyzed and interpreted clinical and pharmacokinetics data, provided critical review of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

We describe a case of attenuated-responsiveness to Artemether-Lumefantrine (AL) in western Kenya. A 4-year old patient with 14% parasitemia was treated with Coartem® but on day 7 still had 5% parasitemia. Parasite genetic profile had similar genetic polymorphism as that selected for when AL is administered. *In vitro* analysis revealed elevated IC₅₀s for AL components compared to the reference *P. falciparum* clone 3D7 and samples collected at this site over the last 5 years. There is need for continued global surveillance to effectively manage emergence and spread of artemisinin drug resistance which has now been found in Southeast Asia.

Keywords: Coartem; resistance; malaria; artemether-lumefantrine; treatment-failure.

1. INTRODUCTION

Artemether-Lumefantrine (AL) is the Artemisinin Combination Therapy (ACT) currently recommended first-line therapy for falciparum malaria since 2006 by Kenyan Ministry of Health (MoH). Artemether -lumefantrine is used in more than 80 countries worldwide and was the first World Health Organization (WHO) prequalified ACT anti-malarial drug, manufactured by Novartis under the brand name Coartem®. Artemether half-life is ~ 1.5 h and lumefantrine is 4 to 6 days, both for the hard and dispersible tablets. AL is administered twice daily for three days as tablets containing 20 mg of artemether plus 120 mg of lumefantrine as follows: 1 tablet (for patients weighing 5–14 kg), 2 tablets (for patients weighing 15–24 kg), 3 tablets (for patients weighing 25–34 kg) and 4 tablets (for patients weighing ≥35 kg). The efficacy of AL has remained > 95% with mild adverse events, most commonly GI (vomiting and diarrhea) and hematologic (anemia and eosinophilia). Although ACTs continue to have excellent cure rates in Africa, there are now confirmed reports of artemisinin-resistance malaria in Southeast Asia [1,2]. A recent report from the coast of Kenya showed that responsiveness to ACTs is declining [3]. This report was the first showing declining responsiveness to artemisinin outside Southeast Asia. The US Army Medical Research Unit – Kenya (USAMRU-K) has an approved active study protocol to study the epidemiology of malaria and drug sensitivity patterns in Kenya. The primary objective of the study is to describe the molecular and *in vitro* drug resistance patterns of *P. falciparum* in Kenya. Consenting/assenting patients who present at

the study sites with symptoms consistent with malaria and are *P. falciparum* positive based on malaria Rapid Diagnostic Test (mRDT) and/or Giemsa stained malaria blood smear (MBS) are enrolled in the study. Here, we report an index case of attenuated-responsiveness to AL treatment after the patient presented with high parasitemia but failed to clear parasites after 7 days.

2. PRESENTATION OF CASE

A 4-year old female patient presented at the Kisumu District Hospital (KDH), one of our study sites with uncomplicated falciparum malaria. The patient was enrolled into the study after it was confirmed that she had malaria infection using the Parascreen® mRDT (Zephyr Biomedical, Verna Goa, India) and Malaria Blood Smear (MBS). The patient was febrile (38.4°C), weighing 13.4 kg with a pulse rate of 156/min and had been symptomatic for two days prior to the clinic visit. No other symptoms were noted. Microscopy analysis of the patient's MBS using absolute white blood cell versus parasite count in 100 high power fields revealed that she had 700,000 parasites/μL. The patient was treated with AL, with health worker directly observing administration of the first dose of 1 tablet with no vomiting. The patient was sent home with the remainder of the dosage with instructions on when to take the drugs. She was instructed to return any time if symptoms persist or on day 7 even if symptoms cease per the study protocol. The patient returned on day 7 as instructed without any obvious clinical symptoms consistent with malaria. However, MBS revealed that she still had 250,000parasites/μL. The

patient's guardian confirmed that the patient completed the dosage as instructed. She received rescue treatment with oral quinine as per Kenya MoH guidelines and was asked to return to the clinic on days 14, 28 and 42. She remained negative for *P. falciparum* by microscopy and PCR in all subsequent visits.

3. MATERIALS AND METHODS

For sample analysis, *Plasmodium falciparum* genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Real-time PCR assay was used for identification of *P. falciparum* as previously described [4]. *P. falciparum* multidrug resistance gene 1 (*Pfmdr1*) copy number was assessed using qPCR as previously described [5]. Polymorphisms in genes encoding *P. falciparum* merozoite surface proteins 1 and 2 (MSP-1 and MSP-2) were used to distinguish between re-infection and recrudescence for samples collected on day 0 and day 7 [6]. Single Nucleotide Polymorphisms (SNPs) in *Pfmdr1* and *P. falciparum* chloroquine resistance transporter (*Pfcrtr*) genes were analyzed using allelic discrimination assay [7] and/or sequenced using 3500xL ABI Genetic analyzer (Applied Biosystems, Foster City, CA). The SYBR Green I-based drug sensitivity assay was used for *in vitro* drug sensitivity testing using a previously described method [8]. Sample isolates collected on day 0 and day 7 were tested against Artemether (AT) and Lumefantrine (LU). *P. falciparum* 3D7 clone which is considered to be chloroquine-sensitive was assayed against both drugs as a reference and internal control.

4. RESULTS

Real-time PCR confirmed that the patient was infected with *P. falciparum* and had a single copy of the *Pfmdr1* gene. Analysis of MSP-1 and MSP-2 showed that day 0 and day 7 samples had the same genetic profiles. Analysis of *Pfmdr1* at codons 86, 184, 1034, 1042 and 1246 revealed the presence of N86, 184F, S1034, N1042 and D1246 genotypes. Analysis of *Pfcrtr* gene at codons 72-76 revealed presence of CVMNK haplotype. *In vitro* analysis revealed the IC₅₀ for artemether/ lumefantrine for the patient on day 0 was 8.94 /58.64 and day 7 was 1.76 / 141.33 nM. Drug concentrations for lumefantrine in plasma collected on day 7 were measured by Agilent HP1100 HPLC machine coupled to CTC PAL autosampler and Waters LC-MS detector

[9]. Prior assay calibration set the lowest limit of lumefantrine quantification at 0.05µg/mL and Limit of detection at 0.025µg/mL. Lumefantrine was below limit of quantification, indicating that a peak was detected but the drug amount was not quantifiable.

5. DISCUSSION

Out of more than 1100 patients recruited at this site since 2008, this was the first case of attenuated-responsiveness to AL treatment. Slow clearance rate of parasites is indicative of artemisinin resistance. Between 2006 and 2011, the median proportion of patients reported to remain parasitemic at 72 h in Africa is 0% and in Asia is 3.9% [1]. Here, we give report of a patient who failed to clear falciparum malaria after taking AL treatment. The first dosage of the treatment was directly observed by the clinician. In addition, the patient's guardian confirmed administering the dosage regimen as instructed. However, lumefantrine drug level was low, indicating the patient might not have complied as instructed. Interestingly, on day 7, the patient was still hyperparasitemic and not symptomatic. Quinine was administered as a rescue second-line treatment to which she responded well.

Genetic polymorphism in *Pfmdr1* along with the mutations in *Pfcrtr* gene confers resistance to different drugs. Specifically, increase in copy number and/or SNPs resulting in an amino acid change in *Pfmdr1* codons 86 (N86Y), 184 (Y184F), and 1246 (D1246Y) have been shown to confer resistance to chloroquine and amodiaquine. In a few studies conducted in Africa, specific polymorphisms in *Pfmdr1* and *Pfcrtr* genes emerged after AL therapy was introduced [10-13]. AL had the opposite effect as chloroquine and amodiaquine on these loci; AL selected for the *Pfmdr1* N86, 184F, D1246 and *Pfcrtr* K76 alleles whereas chloroquine and amodiaquine selected for the *Pfmdr1* 86Y, Y184, 1246Y and *Pfcrtr* 76T alleles. Parasites from this case study carried the same genetic profiles in *Pfmdr1* and *Pfcrtr* genes as that from previous studies showing the emergence of reduced AL susceptibility after a single use of AL [10-13].

The isolate from this patient exhibited slightly elevated IC₅₀s for artemether and lumefantrine compared to samples collected at this site over the last 5 years. In comparison, the IC₅₀s for artemether and lumefantrine for 3D7 reference clone were 4.05 and 40.52 nM respectively.

Efficacy studies in Southeast Asia have shown a clear correlation between parasite genetics and parasite clearance rates, [2,14] some of which are distinct but sympatric parasite subpopulations with high levels of genetic differentiation [15]. Although Southeast Asia has been the epicenter of most anti-malarial drug resistance, studies suggests multiple and independent origins of drug resistance for medications such as chloroquine, sulfadoxine and pyrimethamine [16]. The western part of Kenya was also the first place where the chloroquine resistance was recorded in Africa [17]. ACTs are the first-line treatment recommended for uncomplicated falciparum malaria in most parts of the world. Since studies are already showing distinct parasite subpopulations with different parasite clearance rates which is indicative of multiple and independent origins of this phenotype, more artemisinin resistance efficacy studies outside Southeast Asia are needed. In addition, genetic signatures that are responsible for artemisinin resistance in Southeast Asia might not be the same as those that have emerged or are likely to emerge in other regions.

Based on the analysis of lumefantrine drug level, it is likely that the patient did not completely adhere to drug regimen as instructed. This would have resulted in sub-therapeutic level which can explain the presence of a large number of parasites on day 7. Difficult access to health facilities especially by the poor people living in remote regions, hampering accurate diagnosis and punctual treatment have for decades compromised efficiency of case management [1]. In the present day, this gap has been closed up by community-based care providers with RDTs for prompt diagnosis and ACT treatment under a "test to treat" policy [2,3]. Re-emergence of parasitemia in a subject whose initial dose was supervised by our study clinician identifies the lack of adherence to treatments as an emerging challenge to ACT. Furthermore, it is worth noting that development of resistance occurs in two phases; the first phase is the de novo mutations which are genetic events that produce a resistant mutant and the second phase is selection for the resistant parasites [18]. Presence of sub-therapeutic drug level can increase the selection process resulting in accelerated emergence of drug resistant parasites.

6. CONCLUSION

This case highlights the challenges associated with malaria control and development of drug

resistance. As governments continue to ensure availability of antimalarial treatment, emphasis should be laid on adherence as a way of reducing the rate of development of resistance.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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