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Treatment failure due to the potential under-dosing of dihydroartemisinin-piperaquine in a patient with *Plasmodium falciparum* uncomplicated malaria

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ABSTRACT:

Background: Dihydroartemisinin/piperaquine (DHA-PPQ) 40/320 mg is approved for the treatment of *Plasmodium falciparum* uncomplicated malaria. Different recommendations are provided by WHO guidelines and drug data sheet about dosing in overweight patients. We report here a treatment failure likely caused by sub-optimal dosing of dihydroartemisinin-piperaquine in a case of uncomplicated *P. falciparum* malaria in a returning traveler from Burkina Faso.

INTRODUCTION

Dihydroartemisinin/piperaquine (DHA-PPQ) 40/320 mg tablet formulation is approved for the treatment of *Plasmodium falciparum* uncomplicated malaria in adults and children > 6 months and > 5 kg of body weight. Following WHO guidelines, the daily dose must be proportioned to the body weight and 200/1600 mg (5 tablets) must be administered in patients > 80 kg[1]. Nonetheless according to the drug data sheet, in individuals between 75 and 100 kg of body weight, the recommended daily dose of DHA-PPQ is 160/1280 mg (4 tablets) with lack of data in people over 100 kg of body weight[2]. DHA-PPQ is typically dosed proportionally to the body weight of the patient with a maximum recommended daily dose of 160/1280 mg (4 tablets) according to manufacturer's recommendations[2]. However, WHO identified from pharmacological studies that the standard dosing regimen was inadequate to maintain a therapeutic dose of active compound for individuals in the higher body weight range (typically those > 80 kg). They, therefore, provided an updated dosing body weight dosing schedule in their 2015 guidelines for malaria treatment that provides for a dose of 200/1600 mg (5 tablets) in individuals > 80 kg[1].

CASE REPORT

We report the case of a 54-years old female patient, with a body weight of 86 kg, who was admitted to the Hospital "Borgo Roma" in Verona, Italy, because of fever and dizziness two weeks after returning from travelling in Burkina Faso without using any anti-malaria prophylaxis. Upon presentation at the hospital, a standard blood smear was taken for microscopic examination that diagnosed an infection with *Plasmodium falciparum* trophozoites at a parasitaemia of greater than 2%. A treatment regimen of four tablets of dihydroartemisinin-piperaquine 40/320 mg (total dose 160/1280 mg) taken orally was immediately begun and continued daily for three days. We note that the patient had no vomiting following each dose.

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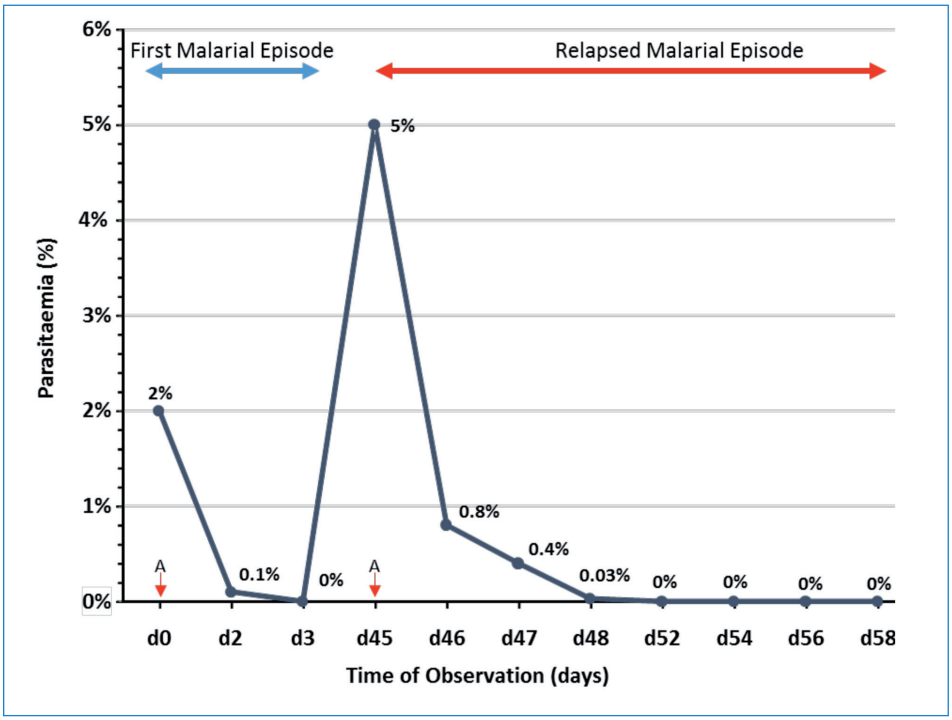

Figure 1. Patient *Plasmodium falciparum* parasitaemia (% Red Blood Cells) over time (days). Two malaria episodes are indicated by the bars above the figure. Hospital admission 'A' is shown by the arrows on the X-axis.

Clinical improvement was observed for symptoms and for parasitaemia followed by blood smears. Parasitaemia was determined negative on the third day of treatment. However, 45 days later the patient was admitted to the Amedeo di Savoia Hospital in Torino (without any further travel to malaria endemic countries) with fever and loss of consciousness. Microscopic examination of a blood smear identified *Plasmodium falciparum* trophozoites at a parasitaemia of greater than 5%. The patient also presented with confusion and aphasia, which led us to undertake a brain computerized tomography (CT) scan with negative findings. Neurological assessment was completed with a brain magnetic resonance imaging (MRI) that revealed a recent 9 mm haemorragic lesion located in the inferior frontal gyrus white matter. A follow-up over time of this lesion was started according to neurological and neurosurgical evaluation and repeated brain MRI scans were unchanged supporting the diagnosis of cerebral cavernoma. Due to the patient's recent history of a *Plasmodium falciparum* infection, it was suspected that this new episode of malaria infection was due to treatment failure from either pharmacological resistance or inadequate drug exposure[1]. A new anti-malarial regimen was started using 200 mg of artesunate administered intravenously (IV) at Time (T) = 0, 12, 24, 48 and 72 hours plus a loading dose of clindamycin (900 mg) IV at T=0 and then 450 mg clindamycin iv *ter in die* (TID) for the next four days. Parasitaemia was monitored by microscopic examination of blood smears daily and was seen to steadily decrease until it became negative on day 4. Treatment was completed with atovaquone/proguanil 250/100 mg for three days. Blood samples daily taken during the second malarial episode have been further analyzed, in order to investigate the possible pharmacological resistance of the *P. falciparum* strain. As previously described, regions from six genes (*PfK13*, *PfCRT*, *PfMDR1*, *PfDHFR*, *PfDHPS*, and *PfCYTb*) linked to resistance to artemisinin derivatives, quinolones, antifolates-cycloguanil and atovaquone have been submitted for DNA Sanger sequencing analysis[3]. In particular, *PfDHFR* sequenced region has been extended to 1-233 codon region. No point mutations associated with artemisinin, piperaquine and atovaquone resistance were identified. *Pfplasmepsin II* (*PfPM2*, PF3D7_1408000) copy number variation and *Pf3D7_1362500 exo-E415G* point mutation have been recently associated with piperaquine resistance in Cambodia[4,5], but analyses performed in our sample by quantitative polymerase chain reaction (qPCR) and sequencing did not reveal any modification in these genes, suggesting that an under dosing of dihydroartemisinin-piperaquine was the most likely cause of treatment failure. Unfortunately, it was not possible to perform analysis of samples from the first malarial episode, as they were no longer available.

CONCLUSIONS

According to WHO guidelines every case of recurrent malaria must be distinguished between reinfection and recrudescence (i.e. treatment failure). Reasons for treatment failure include inadequate exposure to the drug due to sub-optimal dosing, poor adherence, vomiting, unusual pharmacokinetics, substandard medicines or genuine resistance[1]. Due to the widespread treatment failures to DHQ-PPQ in South-Eastern Asia[6,7], the WHO revised their recommended dosing regimens in 2015[1]. This was based on a review of pharmacokinetic data (6 published studies and 10 studies from the WWARN database; total 652 patients) and on subsequent conducted simulations of piperaquine exposures for each weight group: young children (< 25 kg) had a higher risk of lower exposure and treatment failure. Pharmacokinetic data also suggested that patients above 80 kg of body weight may have a similar risk and for this reason, increased weight proportioned daily dosages of dihydroartemisinin/piperaquine

are now recommended (for example, for patients > 80 kg: 200 mg DHA +1600 mg PPQ). The failure of DHQ-PPQ treatment in the patient presented here without any known identifiable mutations in associated gene is similar to other case reports from Africa for DHQ-PPQ treatment failure[3,8]. The first case was an Italian tourist who rapidly responded to DHA-PPQ treatment showing an apparent complete recovery within a few days. However, 32 days after the end of therapy she presented with a recrudescence. Pharmacokinetic analysis had shown that serum concentrations of DHA-PPQ were adequate. Genotyping analysis demonstrated that the same *P. falciparum* strain was responsible for the both episodes and the lack of mutations in the *PfK13* gene suggested the involvement of an artemisinin-sensitive strain. Taken together, these gave support to a hypothesis for resistance to PPQ. The second case occurred in a foreign-born patient living in Italy. Thirty days after malaria recovery following DHA-PPQ therapy, the patient presented with a recrudescence. In this case genotyping analysis not only showed the same strain of *P. falciparum* was responsible for both episodes but also identified similar point mutations to those reported by Gobbi et al[3]. A further case associated to underdosage of DHA-PPQ has been described in a French patient returning from Djibuti[9]. In this case no mutation was found in the *PfK13* gene although *PfMDR1 and PfCRT* polymorphisms were identified. Nonetheless since the body weight of the patient was 104 kg and the patient received a total daily dose of 160 mg dihydroartemisinin (DHA) plus 1280 mg piperaquine tetraphosphate (PPQ), the administered dose was below the WHO recommended ranges. The authors concluded that treatment failure was due to a suboptimal dosage of DHA-PPQ

While both WHO and the manufacturers provide DHA-PPQ dosing for individuals with a body weight above 80 kg, the WHO dosing is greater (200/1600 *vs.* 160/1280) to account for changes in pharmacokinetics of the drugs with weight increases[1]. It should be noted that are the maximum doses provided by the WHO and manufacturer's regimens and do not provide any increase in dose for individuals > 100 kg.

Therefore, in the absence of any associated relevant genetic markers for drug resistance, it is more than likely that the cases presented here and other reported cases have failed DHA-PPQ treatment due to under dosing. In our opinion, there is a need to investigate the dosing for higher body weights (> 80 kg) and provide some guidelines for treatment with DHA-PPQ to prevent recrudescence as a result. In the meantime, besides higher dosages for patients weighing more than 80 kg, close monitoring should be advised in order to early identify relapses and prevent serious complications.

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Conflict of Interests:
The authors declare that they have no conflict of interest.

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