Pretreatment Blood Concentrations of Chloroquine in Patients with Malaria Infection: Relation to Response to Treatment

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Summary

Resistance of *Plasmodium falciparum* to chloroquine has been reported in many areas in Ghana. Most of these reports, which are from hospital-based studies, indicate RI and RII rather than RIII type of resistance. Since high pretreatment levels of chloroquine have also been measured in patients with malaria infection in Ghana, we hypothesized that the 'added effect' of the pretreatment ingested drug to the full dose given at the hospital may be responsible for the low proportion of RIII type of resistance observed. To ascertain this, pretreatment blood levels of chloroquine were correlated with treatment outcomes in 231 paediatric malaria patients, referred to a major hospital in Ghana. The rate of parasite clearance and prevalence of recrudescence, 14 days post-treatment, were determined for each patient. Results from this study showed no correlation between pretreatment chloroquine levels and day 0 parasitaemia. Two hundred and seven patients (89.6 per cent) had parasites that were sensitive to chloroquine whilst 24 (10.4 per cent) had resistant parasites. Of the latter group 17, six, and one patients had *P. falciparum* parasites, which were resistant at RI, RII and RIII levels, respectively. Seventy-five per cent of the patients without any detectable pretreatment blood chloroquine had parasites that were sensitive to chloroquine whilst 89.8 per cent, 98 per cent, and 100 per cent with pretreatment blood chloroquine concentration ranges of 0.5–100.5 ng/ml, 100.5–200 ng/ml, and >200 ng/ml, respectively, had chloroquine-sensitive parasites. An inverse relationship was thus observed between pretreatment blood chloroquine concentration and the degree of resistance in this study. We conclude that pre-hospital treatment ingested chloroquine contributes significantly to the resolution of malaria in children in Ghana, in the presence of chloroquine resistance.

Introduction

Malaria control in Ghana is largely based on chemotherapy using chloroquine, which is the first-line antimalarial drug for the treatment of acute uncomplicated falciparum malaria. The drug is cheap, affordable, easily accessible, easy to administer with few tolerable side-effects, and is routinely prescribed by outpatient clinics to treat 'fever'. It is also the only drug often kept at home by families for home-treatment of malaria being promoted under the Roll Back Malaria Programme (RBM). Home management of malaria (HMM) is a key strategy of providing access to prompt, appropriate, and effective treatment especially for children in sub-Saharan Africa. In Ghana, treatment of uncomplicated malaria is often not sought at health facilities; self-treatment with chloroquine at home is very common,

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but often inappropriate or delayed. This situation could account for the high pretreatment chloroquine concentrations measured in malaria patients in Ghana.\(^1\)

Chloroquine-resistant strains of *Plasmodium falciparum*, first observed in Ghana during 1987,\(^2\) is now widespread throughout the country. Since the first report, others have also reported the existence of significant levels of drug-resistant malaria in various parts of the country (Koram KA, Quashie NB, Duah NO and Abuaku B, unpublished data).\(^3\) Most of these reports indicate parasite resistance at the RI level with a few RII and rarely RIII types of resistance.

Reasons for the low proportion of a high degree (RIII) of parasite resistance observed in these studies, despite the long period since the emergence of chloroquine-resistant malaria parasites, remain speculative. Some have argued that since significant levels of pretreatment blood chloroquine concentration have been measured in malaria patients in Ghana, the ‘additive effect’ of the former to the full dose often given at the health facilities may be responsible for the low proportion of recrudescence at the RIII level. In order to test this hypothesis, pretreatment blood chloroquine concentrations in malaria patients were measured and correlated with treatment outcomes using the standard WHO method for *in vivo* drug sensitivity testing.\(^4\)

### Materials and Methods

Ethical clearance for this study was obtained from the institutional review board of the University of Ghana Medical School.

**Study site and population**

All the children were recruited from the Department of Child Health (DCH), Korle Bu Teaching Hospital in Accra, Ghana. As a policy of the department at that time, children were treated promptly with a full course of chloroquine once it was established that they had malaria. Patients less than 12 years of age were selected for this study after informed consent had been obtained from their parents.

### Drug Treatment

All patients were treated with chloroquine according to the standard regimen of a total of 25 mg/kg body weight given orally as 10 mg/kg initially, then 5 mg/kg 24 hourly starting 6 h after the first dose.

### Monitoring of parasite clearance

Thick and thin Giemsa-stained blood films were examined daily until parasite clearance, then again on days 3, 7, and 14. Response of the parasites to drug treatment was classified in accordance with WHO 1996 mode of classification.\(^4\)

### Chloroquine assay

Whole blood for analysis of chloroquine and desetylchloroquine was obtained in EDTA tubes by venepuncture before chloroquine treatment. Blood samples were analysed using the method described by Alvan, *et al.*\(^5\) The method involves haemolysis of the whole blood by dilution with 1 per cent diethyl amine. Chloroquine and its metabolites were then extracted with diethyl ether. The ether phase was evaporated and the residue re-dissolved in the mobile phase after which it was injected into the HPLC system. The internal standard used was 4-(4-dimethylamino-1-methylbutyl amino)-7-chloroquinoline.

This was a retrospective study using stored samples from patients enrolled in other malaria studies.

### Results

Two hundred and eleven patients with geometric mean pretreatment chloroquine levels up to 64.2 ng/ml (2.6–953.6 ng/ml) and 20 others with no detectable pretreatment blood chloroquine were successfully followed up for 14 days after oral administration of a fresh full dose of chloroquine. Summary of treatment outcome is shown in Table 1 and Fig. 1. The results indicate an association of high pretreatment blood chloroquine concentrations with a low degree of chloroquine resistance (Table 2). The geometric mean pretreatment blood chloroquine concentration decline in the order of $S > R I > R I I > R I I I$.

### Table 1

<table>
<thead>
<tr>
<th>Pretreatment CQ level (ng/ml)</th>
<th>Geometric mean parasitaemia on day 0 (per μl)</th>
<th>Mean parasite clearance day</th>
<th>Treatment outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ($n = 20$)</td>
<td>98 517</td>
<td>2.6</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>0.5–50 ($n = 77$)</td>
<td>81 540</td>
<td>2.8</td>
<td>66 (85.7%)</td>
</tr>
<tr>
<td>50.5–100 ($n = 69$)</td>
<td>48 835</td>
<td>2.6</td>
<td>62 (89.8%)</td>
</tr>
<tr>
<td>100.5–200 ($n = 54$)</td>
<td>98 908</td>
<td>2.7</td>
<td>53 (98%)</td>
</tr>
<tr>
<td>$&gt;$200 ($n = 11$)</td>
<td>99 650</td>
<td>2.1</td>
<td>11 (100%)</td>
</tr>
</tbody>
</table>

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The patients were also categorized into malaria type (cerebral, severe anaemia, and uncomplicated malaria) and the pretreatment blood chloroquine level for each group was determined. Geometric mean pretreatment chloroquine levels were 58.7 ng/ml, 63.6 ng/ml, and 63.2 ng/ml blood for severe malarial anaemia, cerebral malaria, and uncomplicated malaria patients, respectively, while desethylchloroquine levels were 15.2 ng/ml, 14.3 ng/ml, and 36.6 ng/ml, respectively, for the same categories. The ratio of CQ:DCQ was 3.4 for severe malarial anaemia, 4.2 for cerebral malaria, and 1.2 for uncomplicated malaria patients.

The study also correlated pretreatment blood chloroquine levels with the degree of existing parasitaemia on day 0. No direct correlation between the two variables was observed; profound pretreatment blood chloroquine levels of more than 200 ng/ml were equally associated with high parasitaemia (547 840 parasites/μl blood) or low parasite level (280 parasites/μl blood).

Statistical analysis to establish the association between pretreatment blood chloroquine concentration and treatment outcome is shown in Table 2.

![Plot of pretreatment blood chloroquine concentration against treatment outcome.](image)

**Table 2**

<table>
<thead>
<tr>
<th>Pretreatment blood CQ conc (ng/ml)</th>
<th>Treatment outcome</th>
<th>OR (95% CI)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (%)</td>
<td>R (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>75</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>0.5–50</td>
<td>85</td>
<td>15</td>
<td>0.45 (0.22–100)</td>
</tr>
<tr>
<td>50.5–100</td>
<td>90</td>
<td>10</td>
<td>0.33 (0.14–0.78)</td>
</tr>
<tr>
<td>100.5–200</td>
<td>98</td>
<td>2</td>
<td>0.06 (0.01–0.28)</td>
</tr>
<tr>
<td>&gt;200</td>
<td>100</td>
<td>0</td>
<td>0 (0.00–0.15)</td>
</tr>
</tbody>
</table>

*S, sensitive to treatment; R resistant to treatment; OR, Odds ratio.*

Using the EPI-INFO statcalc, the chi-square for the trend in proportions was determined. A chi-square of 10.7 with a p-value of 0.001 was obtained. Odd ratios for resistance were calculated as 1.0, 0.5, 0.3, 0.06, and 0 for pretreatment blood chloroquine concentration ranges of 0 ng/ml, 0.5–50 ng/ml, 50.5–100 ng/ml, 100.5–200 ng/ml, and >200 ng/ml, respectively (Table 2).

These values demonstrate that the trend observed in this study is statistically significant.

**Discussion**

This study demonstrated an association between whole blood concentrations of pre-hospital treatment chloroquine and the resolution of malaria following treatment of the disease with chloroquine. Observations made in this study indicate that pretreatment blood chloroquine concentration has an inverse relation with the degree of *P. falciparum* resistance to chloroquine. This suggests that, high pretreatment blood chloroquine assists in eliminating chloroquine resistant strains of the parasites during drug treatment and thus plays a role in the observed reduced proportion of RIII-type resistance in Ghana. This observation may probably be due to the ‘added effect’ of the pretreatment ingested drug.

When a reference minimum inhibition concentration (MIC) of 90 ng/ml for *P. falciparum in vivo* is used, the indication is that the levels of pretreatment whole blood chloroquine measured in this study are significantly high. This means that pretreatment blood chloroquine may significantly affect the initial parasitaemia as it adds up to the effect from the full dose given at the hospital.

The beneficial role of pretreatment ingested chloroquine as shown in this study is good news for the national malaria control programme since they have been encouraging home management of malaria (HMM) under the Roll Back Malaria programme.

Our finding presupposes that a child who receives treatment with chloroquine at home before hospital treatment will have a better chance of resolving
the disease sooner than later. This phenomenon had already been discussed.7

Deductions from this study indicate that combination therapy is being practised indirectly in Ghana. If parents who practice HMM with chloroquine are compelled to take their children to hospital due to delayed resolution of the malaria they are more likely to indirectly receive combination therapy if their children are put on an alternative antimalarial drug such as sulfadoxine/pyrimethamine or artemisinin derivatives. The potential value of malaria therapy using combinations of drugs has been identified as a strategic and viable option in improving efficacy and delaying the development and selection of resistant parasites.8 However, in the present study, we did not examine the impact of pre-hospital chloroquine on the resolution of malaria following treatment with other antimalarials such as artemisinin derivatives or sulfadoxine/pyrimethamine, both of which are commonly prescribed for the management of malaria in Ghana.

Current policies of World Health Organization recommend that treatment policies for falciparum malaria in all countries experiencing resistance to monotherapies should be combination therapies, preferably an artemisinin-based combination therapy (ACT). Artemisinin-based combination therapies have been shown to improve treatment efficacy and to delay drug resistance in south-east Asia.8–10

In order to halt the devastating effect of the disease due to the reduced susceptibility of the parasites to chloroquine in Ghana, the National Malaria Control Programme (NMCP) is proposing a change in antimalarial drug treatment policy. A combination of antimalarial drugs, possibly amodiaquine with artemunate, has been proposed to replace chloroquine as the first-line antimalarial drug (Antimalaria Drug Treatment Review Taskforce, personal communication).

Overall, more than 85 per cent of all categories of patients involved in the study had detectable pretreatment blood levels of chloroquine and its metabolite, desethylchloroquine. Geometric mean chloroquine concentrations of 61.1 ng/ml blood and 64.2 ng/ml blood were measured for patients with severe malaria (severe malarial anaemia and cerebral malaria) and uncomplicated malaria, respectively. This study could not, therefore, demonstrate any significant difference in pretreatment blood chloroquine levels among the different categories of malaria patients. However, it must be emphasized that the detection of high pretreatment blood levels of chloroquine and its metabolite in most of the patients shows the widespread use of the drug in Ghana. Although attempts are often made in hospitals and clinics to obtain a history of drug intake before treatment, the information given is usually not certain enough for clinicians to withhold or delay treatment of severely ill children. Our concerns are for those with extremely high pretreatment blood chloroquine who still have to receive a further dose of the drug. During treatment, a clinician should not only aim at dosing patients to attain adequate therapeutic blood chloroquine level that will resolve the malaria, but should also have in mind the possibility of the patient obtaining an overdose of the drug and the consequent toxic effect.

In Ghana, the adverse effect of chloroquine is poorly documented due to reasons such as limited availability of equipment to monitor drug levels and the difficulty and unreliability of clinical diagnosis alone for chloroquine intoxication. To eliminate chloroquine intoxication, another antimalarial could be recommended if initial investigation indicates that patients have already ingested some chloroquine. It would therefore be useful if a rapid test for blood chloroquine levels was performed before initiation of treatment. It will also be necessary to determine post-mortem chloroquine levels in children who die from an initial malaria attack, so as to establish the role of chloroquine toxicity in the cause of death of children admitted to the referral hospitals.

The CQ:DCQ ratio, which is an important indicator of the extent of chloroquine metabolism, was high, even among patients with RII-type resistance, as stated above. This confirms the existence of high-prevalence chloroquine resistance in Ghana.

In conclusion, this study has demonstrated the existence of chloroquine resistance P. falciparum malaria in Ghana, the severity of which is curtailed by the beneficial role (added effect) played by pre-hospital treatment ingested chloroquine.

References
7. Dunyo SK, Afari EA, Koram KA, Ahoorlu CK, Abubakar I, Nkhumah FK. Health centre versus

