Therapeutic Efficacy of Chloroquine in Plasmodium Vivax at Health Centers in Jimma Town, South-West Ethiopia

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Abstract
Introduction: - Plasmodium vivax accounts for about 40% of all malaria infection in Ethiopia. Chloroquine remains the drug of choice for the treatment of *p.vivax* malaria in the country. Emerging resistance to chloroquine (CQ) by *P.vivax* threatens the health of the hundreds of thousands of people regularly exposed to the risk of infection with this organism. There was 14 years back failure alarm report of *P.vivax* to chloroquine but there has been no research in Jimma town which show extent of efficacy of medication. Therefore this study designed to assess the therapeutic efficacy of chloroquine used in the treatment of *P.vivax* malaria infection at health centers in Jimma town South West Ethiopia.**Methods**: An in vivo prospective drug efficacy study was conducted in Jimma town from February10 to May 09, 2011. Eight one Patients with microscopically confirmed *P. vivax* malaria, aged between 6 months and 60 years, were recruited and treated under supervision with Chloroquine (10mg/kg at 0 and 24 hrs followed by 5mg/kg at 48 hrs orally). Clinical and parasitological parameters were assessed during the 28 day follow-up period as per with methods for surveillance of antimalarial drug efficacy WHO 2009. Data was analyzed using SPSS, version 16.0.**Results**: Of the total 81 patients included in the study, 74 completed their 28-day follow-up. Despite of the 100% clearance of both fever and parasitemia on day 3, parasitaemia reappeared in two participants within the 28-days follow-up with the absence of malaria symptoms. Therefore, the cumulative incidence of treatment failure was 2.7% (with 95%CI - 0.99 - 6.39) in study participants. **Conclusions**: chloroquine is still now efficacious drug to *P.vivax* in study area. However, reappearance of the parasite within the 28 days of follow-up in the study area signals the need for launching monitory activities for Chloroquine resistant by the *P. vivax* parasite.
Keywords: malaria, *p.vivax*, chloroquine, therapeutic efficacy, Jimma.

INTRODUCTION

Malaria is a protozoan disease transmitted by the bite of infected Anopheles mosquitoes. Transmission by a transfusion of parasite infected blood and transplacental transmission are also possible (White NJ *et al*., 2009).

Malaria remains a major global problem, exacting an unacceptable toll on the health and economic welfare of the world's poorest communities. There were an estimated 247 million malaria cases, (189–327 million) worldwide, of which the vast majority of cases (86%) were in the African Region. There were an estimated 881, 000 (610,000–1, 212, 000) deaths worldwide, of which 90% were in the African Region (WHO, 2008).

In Ethiopia, malaria is ranked as the leading communicable disease accounting for about 30% of the overall disability adjusted life years lost (mop EFY, 2010). In 2007/2008, malaria was the first cause of outpatient visits, health facility admissions and in-patient deaths, accounting for 12% of out-patient visits and 9.9% of admissions (Daddi J *et al.,* 2010).

Plasmodium vivax is neither rare nor benign. It occurs throughout the tropics, except in western and central sub-Saharan Africa. It is the most geographically widespread and the second prevalent cause of malaria globally. About 10–20% of the global burden of *p.vivax* malaria occurs in Africa, especially in Eastern and Southern African countries. In Ethiopia, *P. vivax* malaria accounts for 40% of whole malarial case (Price NR *et al.,* 2007 and EFMOH, 2004).

Early treatment is important to prevent severe illness and death due to malaria. Currently, chloroquine has been used as first-line drug for treatment of *P. vivax* infection throughout the Ethiopia as well as most part of the world. Historically, chloroquine clears fever and parasitemia caused by *P. vivax* within, at most, 72 h of the first dose. The drug is very rapidly absorbed and slowly eliminated, principally as a parent drug and a desethyl metabolite in roughly 3:1 proportions. The plasma half-life is about 50 h, and therapeutic levels against *p.vivax* malaria persist in blood until about days 21 to 35 after the start of treatment (EFMOH, 2004 and Rosenthal PJ *et al*., 2008).

Emerging resistance to chloroquine (CQ) by *p.vivax* malaria threatens the health of the hundreds of millions of people routinely exposed to the risk of infection with this organism. Resistance by *P. vivax* was unknown until 1989, when Australians repatriated from Papua New Guinea failed routine treatment (Baird JK et al., 2004). Since then there were many reports and researches confirming the resistance of *P.vivax* to chloroquine.

In Ethiopia there was a report of failure alarm for chloroquine 14 years back. In 2003 a study in

Debrezeit and Nazareth, Ethiopia showed four treatment failures among 135 patients who completed 28 days follow up period in CQ treated group without determining plasma drug concentration (Asnakew KY *et al*., 2010). In year 2006 another in vivo drug efficacy study conducted in Debre Zeit, Ethiopia, showed 4.6% cumulative incidence of therapeutic failure within 28 days of follow up period for standard directly supervised dose of chloroquine therapy (Hiwot T *et al*., 2008). More recently, a similar study conducted in Serbo town, Jimma zone, Ethiopia showed 3.6% therapeutic failures (Tsige K *et al*., 2009). However, such efficacy study is totally absent in jimma town despite of being one of major malarious site of the country. Therefore, as aimed to assess the therapeutic efficacy of chloroquine against *P.vivax* malaria at public health facilities in Jimma Town, this study revealed 2.7% treatment failure.

METHODS

Study area and period

The study was conducted in Jimma town at two health centers from February 10 to May 09, 2011. Jimma town is located 350Km south-west of Addis Ababa. The town's geographical coordinates are approximately 7°41' N latitude and 36° 50'E longitude. The town is found in an area of average altitude of about 1780 m above sea level. It lies in the climatic zone locally known as Woyna Daga which is considered ideal for agriculture as well as human settlement. The town is generally characterized by warm climate with a mean annual maximum temperature of 30°C and a mean annual minimum temperature of 14°C. The annual rainfall ranges from 1138 mm to 1690 mm.The town had the total population of 120,600 in 2007 population census and the majority of the population is Oromo by ethnicity. Jimma town comprised of one governmental specialized hospital, three health centers and nongovernmental many private clinics, pharmacies, drug stores and drug venders.

Study design

The study design was one-arm prospective evaluation of clinical and parasitological responses of directly observed chloroquine treatment of uncomplicated *p.vivax* malaria per WHO 2009 protocol.

Study participants

Suspected patients seeking medication in Jimma Health Centers were examined for the presence of *P. vivax* parasite monoinfection, on thick and thin film preparations, witha threshold of 250 parasites/µl or above. Among the screened patients, those who fulfilled inclusion criteria set by WHO (WHO 2009) were recruited for the 28-days *in vivo* study.

The sample size was calculated based on the expected proportion of *P. vivax* treatment failures with CQ in the study population as recommended by WHO protocol 2009 (WHO 2009) on assessing antimalarial drug efficacy and safety. Assuming a maximum of 4.6% treatment failure in the population, at a confidence level of 95% and margin of sampling error tolerated 5%, minimum sample sizes was 67 and with 20% increase to allow loss to follow-up and withdrawals during the 28 day follow-up period, a total of 81 study participants were enrolled using Quota sampling technique.

Data collection procedure

Treatment and follow-up

Patients with uncomplicated *P.vivax* malaria, who met the inclusion criteria were enrolled, treated on site with Chloroquine (10mg/kg at 0 and 24 hrs followed by 5mg/kg at 48 hrs orally) and monitored for 28 days.

Clinical evaluation

All patients were evaluated clinically by health officers.Physical examination were performed at the baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, and 28. A demographic information and contact details, complete medical history including malaria signs and symptoms and medication history were screened at baseline in outpatient department. Medical and medication history were being assessed continuously on each patient visit.Body weight was recorded on day 0 to calculate the dose of drug to be administered. Axillary temperature was measured at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21 and 28. Temperature was measured with thermometer that has a precision of 0.1°C.

Parasitologic investigation

For blood collection, finger-prick blood samples were collected from consenting patients for malaria parasite identification by four laboratory technicians two from each health center.

Thick and thin blood films were prepared and stained with 10% Giemsa stain for parasite counts and examined for screening on day 0 to confirm adherence to the inclusion and exclusion criteria. Thick blood films were also examined on days 2, 3, 7, 14, 21, and 28. Parasite count was based on the number of asexual parasites observed against 200 leukocytes. Parasite density calculated as:

$$\text{Parasite density (per µl)} = \frac{\text{Average number of parasite counted x (6000-8000) WBCs}}{\text{Average number of leukocytes counted}}$$

Quality assurance

For microscopic investigation a fresh Giemsa stain dilution was prepared at least once a day. For drug qualities a nationally recognized source of quality drugs was used: - Chloroquine phosphate 250mg tablet equivalent to

150mg chloroquine, batch number 0040223, Ethiopian pharmacietical manufacturing.

The principal investigator ensured that the study protocol was strictly adhered to and that all data were being collected and recorded correctly on the case report form. Laboratory and clinical data were recorded on a daily basis on the case report form designed for the study. The principal investigator was responsible for keeping all screening forms, the case report form and the completed subject identification code list in a secure location. After completion of data collection, data were entered into a SPSS database by double independent data entry.

Data Analysis

Data collected from *in vivo* therapeutic efficacy test was double entered and analyzed using SPSS (version 16.0). Descriptive statistics were used for demographic and clinical characteristics of participants at day of enrollement. Kaplan-Meier survival probability analysis was used to evaluate treatment outcome of study participants during follow-up period. The proportion of patients who experienced therapeutic failure during the follow-up period was used to estimate the efficacy of study drug. Other statistics used were Pearson's correlation for correlation between parasite and fever clearance time, logistic regression to test association between age; and sex and treatment response and Mann-Whitney u test to compare difference in geometric mean parasite between treatment failures and adequate clinical and parasitological responses.

Ethical considerations

Approval by the Jimma university ethical review committee was obtained before the study.

Patients were included in the study only when they or guardians in the case of children gave informed consent. All patients' information was remaining confidential and was shared only by the study team.

RESULTS

Patients who were febrile or had history of fever with in past 24 hours of diagnosis were sent from out patient department to laboratory and diagnosed for malaria. Among patients who were *P.vivax* positive, a total of 81 eligible patients were enrolled. Among 81 enrolled 5 were lost to follow up, 2 were withdrown from study and 74 were completed 28 days of follow up. Among 81 participants 60.5% were adults and 41(50.6%) were male (table 1).

Among the study participants, temperature was range from 36 to 40.3°C and parasite load range from 1200 to 15600count/ µl on the day of enrollment. Descriptive results are presented below.

In age and sex distribution of clinical characteristics, relatively higher geometric mean parasite load and mean axillary temperature was recorded in underfive age group while relatively lower in middle age group (table 3).

Gennerally fever and parasite clearance rate look like collinear with in first three days. Then fever curve maintained horizontal while geometric mean parasite was not (figure 1).

There was 98.8% fever clearance and 93.8% parasite clearance on day 2. On day 3 there was 100% clearance of both parasite and fever among 79 participants who continued in the study.

However, we did not detected any significant correlation between fever clearance time and parasite clearance time (Pearson correlation, $r = 0.106$, $P = 0.347$).

According to Kaplan Meier analysis the total cumulative incidence of treatment failure was 2.7 %(95% CI: -0.99 - 6.39). Since exclusion from study in seven participants had ocured (on day 2 and day 3) before treatment failure (on day 7 and day 28) censoring for analysis does not alter the cumulative incidendence of treatment failure. Exclusion from study is due to self administration of antimalarial drug in two patients and loss to follw up in five participants.

We did not detect a significant difference in geometric mean parasite density at enrollment between treatment failures (mean rank, 41.75) and successes (mean rank, 37.38; Mann-Whitney U test, $P = 0.777$).

DISCUSION

Chloroquine clears fever and parasitemia caused by *plasmodium vivax* within, at most, 72 h of the first dose. Historically, it was 100% effective in treatment of *p.vivax* (Rosenthal PJ 2008). This study revealed 97.3% effectiveness of the drug at the end of 28 days follow up.

In this study, at the day of enrollement 82.7% of patients were fibrile and 98.8% cleared within 48 hours and 100% within 72hours of the follow up. Parasite clearance rate was 93.8% on day 2 and 100% on the day 3. This is almost similar with a study which was conducted in Debre Zeit (Hiwot T *et al.,* 2008), 98% parasite clearance on the day 3 and has higher response rate than a study which was conducted in Serbo town in Jimma Zone (Tsige K *et al*., 2009) in which parsite clearance at day 3 was 88% while fever clearance was 89.7%.

Eventhough there was 100% clearance rate of both fever and parasitemia within 72hours of follow up; there was insignificant correlation between fever and parasite clearance (Pearson correlation, $r = 0.106$, $P = 0.347$). This may indicate fever may not depend up on parasite load only but also individual variation of body response to the parasite may be important. The faster fever clearance rate may be due to paracetamol coadministration in fibrile patients.

In fact medication effect differs with age and sex, but in this study both sex and age group of participants were insignificantly associated with parasite and fever clearance rate of chloroquine in *P.vivax* malaria (table 5). This may indicate efficacy of chloroquine in *P.vivax* malaria is less dependent on age and sex when given equivalent dose adjusted to body weight.

In this study, we were able to observe two late parasitological failures, in an eight years old male participant and 18years old female participant. The male patient on the day of enrollement had axillary temperature of 38.8°c and 8010/µl of parasite load. Fever cleared within 48hours where parasite load decreased to 840count/µl on day 2 and undetectable on the day 3. However, parasitemia reappeared on day 7 without any clinical symptom of malaria.

In the 18years old female patient, on day of enrollment, she had axillary temperature of 37.6°c and fever was cleared within 24hours. Parasite load on enrollement was 3280count/µl and she was cleared of parasitemia within 48 hours. Despite fast clearance of fever and parasitemia, there was reappearance of parasitemia on day 28 without clinical symptom of malaria.

In this study five participants were lost to follow up despite of every effert (two on day 2 and three on day 3 of follow up) and there was two withdrawals (one on day 2 and one on day 3) due to self administration of other antimalarial drug (COARTEM^{R}). Therefore, two participants on day 2 and five participants on day 3 were excluded from study. However, treatment failure was occurred on day 7 and on day 28. In this case, censoring for withdrawal and lost to follow up do not affect cumulative incidence of treatment failure. Therfore, our cumulative incidence of treatment failure was 2.7%.

The similar studies which were conducted in Debre Zeit and Serbo town in Jimma Zone, Ethiopia, were revealed hardly lower response rate. In Debre Zeit the cumulative incidence of resistance was 4.6% and in Serbo town it was 3.6%. Higher response rate in our study than in Debre Zeit and Serbo Town does not implay increasing sensitivity because study sites are different .i.e. resistance is different in different catchment areas. Despite of a little difference in response rate in our study and above two studies, the failures were late parasitological (Hiwot T *et al*., 2008, Tsige K *et al*., 2009). Similar study result from Madagaskar (Barnadas C *et al*., 2008), showed higher failure rate. There was 10.2% recurrence and after genotyping correction, five cases (5.1%) of recurrence were confirmed as resistance.

Eventhough there was relatively high parasite load at day of enrollement (Day 0) in treatment failures; we did not detect a significant difference in geometric mean parasite density at enrollment between treatment failures and successes in Mann-Whitney, $P = 0.777$). Despite of different test statistics, this result was in agreement with a study in Debre Zeit (21) (p=0.061). A similar study in the eastern Indonesia (Sutanto *et al*., 2009) showed similar result ($P = 0.341$). This may indicate recurrence of parasitemia after standard treatment less probably dependas up on parasite load before treatment.

Recurrence parasitemia may be due to different reasons; recrudence, reinfection or reactivation of liver hypnozoit of plasmodium. The day 7 recurrences is most probably resistance strain and less probably reinfection or relapse, because plasma therapeutic concentration sustains more than 21 day after three day standard dosage of chloroquine. Even if it is reinfection or relapse, it is most probably resistance strain. However, our study lacks test of therapeutic level of chloroquine in the blood to say so. Recurrence on day 28 may be due to any of above mentioned reasons which can only be determined genotyping in addition to blood drug concentration. Ufortunately, our study suffers limitation of unavailability of genotyping of *P.vivax* species.

CONCLUSSIONS

In general, this study revealed good clinical and parasitological response of *plasmodium vivax* to chloroquine. Our study also showed fast clearance of fever and parasitemia within maximum of 72hours of start of medication. Therefore, chloroquine can be continued as 1st line medication for the *P.vivax* malaria in the study area. However, reappearance of the parasite within the 28 days of follow-up in the study area signals the need for launching monitory activities for Chloroquine resistant by the *P. vivax* parasite.

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Table I. Age and sex of the study participants on the day of enrollement, Jimma town, southwest Ethiopia, 2011

Characteristics		Value at day of admission (Day 0)
Age groups (years)	< 5	7(8.6%)
	5–14	25(30.9%)
	15+	49(60.5%)
Sex	Male	41(50.6%)
	Female	40(49.4%)

Table II Clinical characteristics of the study participants on the day of enrollement, Jimma town, southwest Ethiopia; 2011

Characteristics	Value at day of admission (Day 0)
Mean Body Temp. in °C ± SD	38.00 (±0.86)
Febrile (Axillary Temp. ≥ 37°C) (No/%)	67 (82.7%)
History of Fever (No. / %)	79 (97.5%)
Geometric mean parasite/µl ± SD	5276 (±3257)

Table III. Base line clinical characteristics of participants stratified by age and sex, in Jimma town, Southwest Ethiopia, 2011

Age in year	sex	Mean body Temp.(°C)	Febrile (Axillary Temp. >37°C) /%	History of Fever within past 24hrs /%	Geometric mean parasite/µl
<5	M	38.4	100	100	5750
	F	37.4	60	100	6566
5-14	M	37.9	81.2	93.7	5158
	F	37.4	66.7	100	3680
>14	M	38.2	91.3	95.5	4892
	F	38.0	92.3	100	5957
All age group	M	38.1	87.8	95.1	5037
	F	37.8	82.5	100	5521

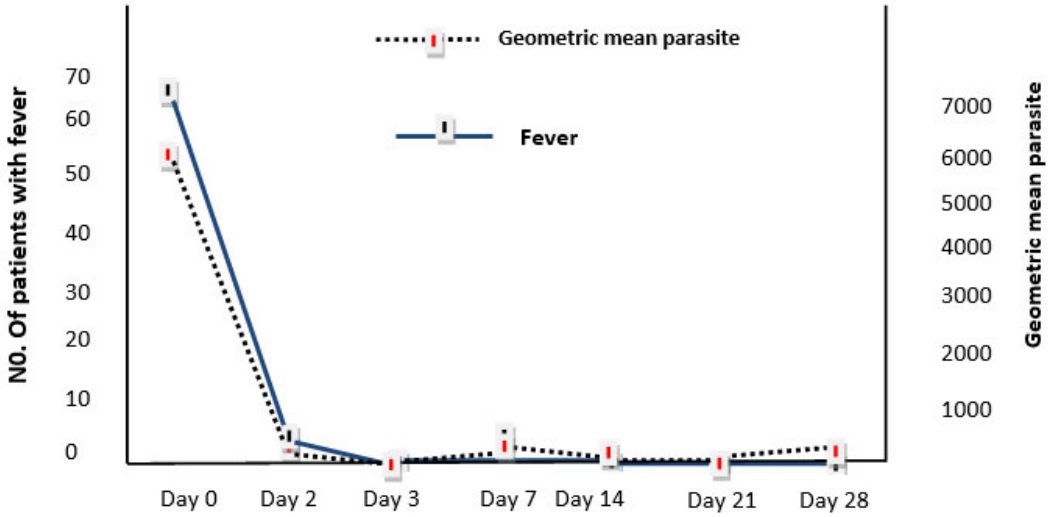

Figure I. General trend of fever and parasitic load clearances in the study participants in Jimma town, Southwest

Table IV. Aassociation between demographic characteristics and treatment response, Jimma town, Southwest Ethiopia, 2011

for fever clearance				
Age and sex	**P value**	**OR**	**95% CI of OR**	
5	0.999	0.000	0.000	+∞
5-14	0.921	1.100	0.166	7.303
>14		1.00		
female	0.244	0.261	0.027	2.506
Male		1.00		
For parasite clearance				
5	0.206	5.726	0.383	85.567
5-14	0.629	1.659	0.213	12.936
>14		1.00		
female	0.181	0.199	0.019	2.119
Male		1.00		

Table V. Treatment outcome of study participants versus days of follow up, in Jimma town, Southwest Ethiopia, 2011

Follow-up Days	PR	W	TF	II	CITF	95% CI	
						LL	UL
Day 0	81	0					
Day 1	81	0	0	0	0	-	-
Day 2	81	2	0	0	0	-	-
Day 3	79	5	0	0	0	-	-
Day 7	74	0	1	0.0135	0.0135	-1.63	3.98
Day 14	73	0	0	0	0.0135	-1.63	3.98
Day 21	73	0	0	0	0.0135	-1.63	3.98
Day 28	73	0	1	0.0135	0.0270	-0.99	6.39

PR=Population at risk, W=Excluded from the study (2 withdrawal and 5 loss to follow up), TF= Treatment failure, II=interval incidence, CI=confidence interval, LL=Lower limit, UL=Upper limit, Confidence interval describes range of probability under which cumulative incidence of treatment failure may fall.