Therapeutic efficacy of chloroquine for the treatment of *Plasmodium vivax* malaria among outpatients at Shawa Robit Health Care Centre, North-East Ethiopia

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ABSTRACT

Nearly 40% of all malaria infection in Ethiopia is caused by *Plasmodium vivax*. Chloroquine (CQ) is the first line treatment for confirmed *P. vivax* malaria in the country. However, the efficacy of this drug has been compromised by CQ resistant *P. vivax* (CRPv) strains. Therefore, the present study was aimed at assessing the therapeutic efficacy of CQ for treatment of *P. vivax* malaria at Shawa Robit Health Care Centre, North-Ease Ethiopia. A one-arm, 28-day follow-up, *in vivo* therapeutic efficacy study was conducted from October 2013 to February 2014. Eighty-seven patients with microscopically confirmed *P. vivax* mono – infection aged between 1 and 65 years were enrolled and treated with a 25 mg/kg *CQ* administered for three consecutive days under supervision. Socio-demographic and clinical information were collected. Blood smears were prepared and examined for parasite clearance or recurrence of parasitaemia. Clinical examination was performed at all follow-up visits. Haematocrit determination was made. Percentages, frequencies, Kaplan–Meier survival probability analysis and statistical associations were computed. *P*-value of <0.05 was considered statistically significant. From the total 87 patients included in the study 76 (87.4%) completed their 28-day follow-up; four patients were excluded due to *P. falciparum* infection during the follow up (on day 2, day 7 and day 14) and seven cases were lost to follow-up (on day 3, day 7 and day 14). Among those *P. vivax* infected individuals, 44 (50.6%) subjects were febrile on day of admission and the remaining had history of fever. From the 76 study participants who completed the 28-day follow up period, late parasitological failure (LPF) was observed in five (6.6%) cases. The geometric mean of parasite density was 8723.9/µl and mean haematocrit value was 35.45%. Besides, survival analysis showed that the cumulative incidence of success and failure rates at day 28 was 93.4% (95% CI = 0.849–0.972) and 7.04% (95% CI = 0.028–0.151), respectively. The current study unveils possible emergence of CRPv malaria in the study area. Regular and periodic evaluation of the efficacy of CQ should be conducted to monitor the spread of CRPv strains.

1. Introduction

Plasmodium vivax has not been given much attention as a public health priority for many years. Nonetheless, nowadays, it has become a global, public health and Socio-economic problem for many countries (Lover and Coker, 2013). The public health impor-tance of vivax malaria is attributable to its ability to cause a range of severe and life-threatening syndromes similar to malaria caused by *P. falciparum* (Genton et al., 2008; Kochar et al., 2009). Unlike *P. falciparum*, a single infection of *P. vivax* may cause frequent illness due to multiple relapses after the primary infection. These weaken adults and affect the growth and schooling of children, thus dam-aging economic development of a country or region (FMoH, 2006; WHO, 2006).

Hence, malaria control strategies are not equally effective for *P. vivax* and *P. falciparum*. This is because of the control strategies in *P. vivax* are affected by factors such as lack of access to reliable diagnosis, early transmission of the parasite in the course of the

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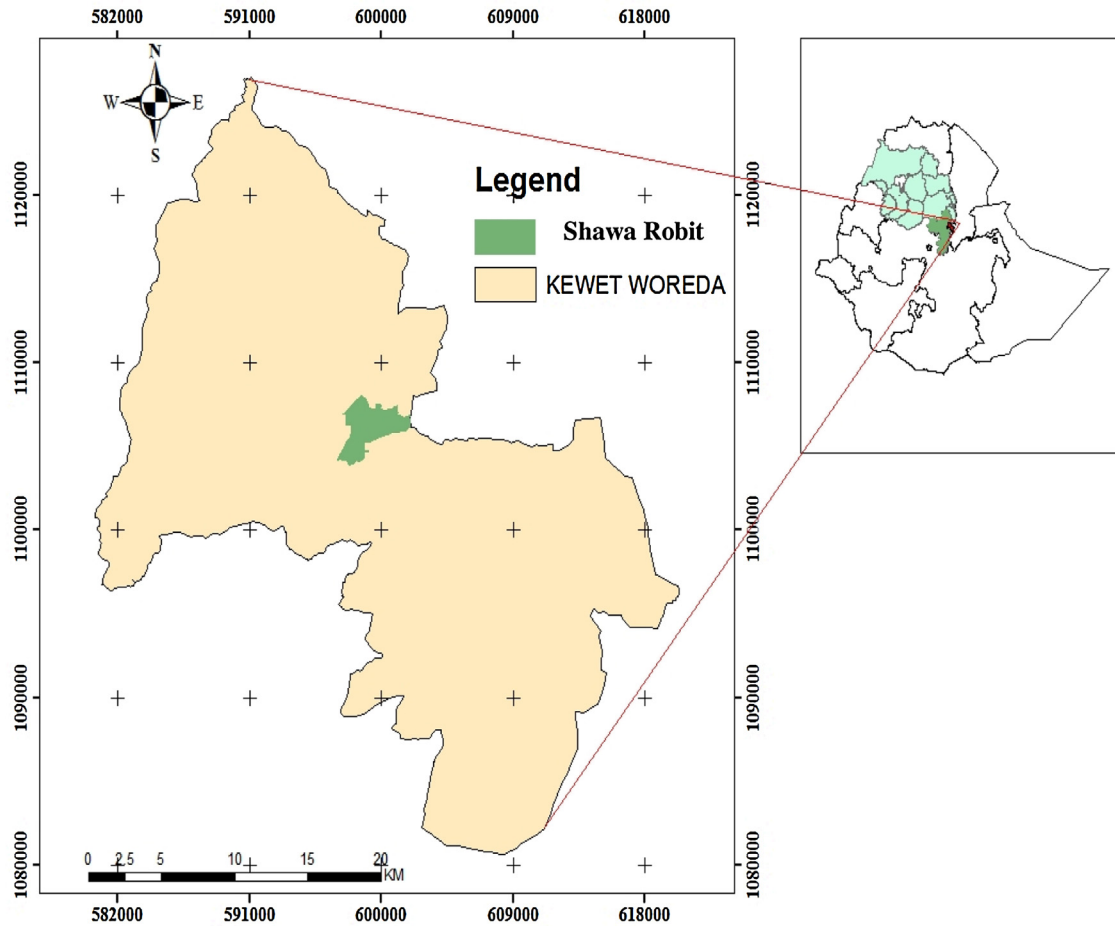
Fig. 1. Map of the study area.

disease, its relapsing nature and geographic variation. Therefore, to reduce the burden of disease, effective treatment of vivax malaria is imperative (Lover and Coker, 2013; Price et al., 2007; WHO, 2011). To resolve complication of malaria in patients and (FMoH, 2006) and spread of drug resistance in the community, effective treatment of malaria is important (Ethiopia).

CQ is the recommended first-line treatment for *P. vivax* malaria in most parts of the world except in few areas with widespread CQ resistance (Harijanto, 2010; WHO, 2010a,b). However, there is emergence and spread of CRPv strains in many parts of the world where CQ is used first line drug including Ethiopia (Ketema et al., 2011; Ratcliff et al., 2007; Teka et al., 2008).

The cost for malaria control has been increased due to antimalarial drug resistance. Therapeutic failure needs further diagnosis and treatment of malaria; consequently it increases the cost of the health system, loss of working days and school absenteeism (Talisuna et al., 2004). Therefore, regular monitoring of CQ efficacy is necessary for early detection of resistance and timely change of the treatment policy of a country. The World Health Organization (WHO) recommends therapeutic efficacy studies to be conducted for first and second line antimalarial drugs at least every two years. Change of treatment policy is recommended when the treatment failure rate is above 10% (FMoH, 2006; WHO, 2001).

Emergence and the spread of CRPv strain has become a major public health problem in different countries, and requires regular monitoring program. Such kind of monitoring should be implemented in countries like Ethiopia where there is limited information on efficacy of CQ. Evidences from efficacy studies may help in obtaining information in order to develop or change treatment policies. Therefore, the current study was aimed at assessing the therapeutic efficacy of CQ for the treatment of vivax malaria among outpatients at Shawa Robit Health Care Centre, North-East Ethiopia.

2. Methods

2.1. Study site and population

The study was conducted in Shawa Robit Health Care Centre, found in Shawa Robit Town, North-East Ethiopia (Fig. 1). The Town is located at 225 km northeast of Addis Ababa, in the Amhara Regional State at an average elevation of about 1280 m above sea level. It is found at longitude of 10°060 N 39°590 E and latitude of 10.1° N 39.983° E. It has a total population of 42,208. The area receives high rainfall during the main rainy season (June to September) and is characterized by markedly unstable seasonal malaria (FMoH, 2006).

The study participants were individuals who had confirmed *P. vivax* mono-infection on thick and thin blood film preparations and who fulfilled the inclusion criteria (WHO, 2009). The source population was those clinically malaria-suspected individuals with fever or history of fever and seeking treatment at Shawa Robit Health Care Centre during the study period. The inclusion criteria includes age over 6 months, mono-infection with *P. vivax* detected by microscopy, asexual parasite count >250/µl, axillary temperature ≥37.5 °C or history of fever during the 48 hs before recruitment, ability to swallow oral medication, ability and willingness to comply with the study for the duration of the study and to comply with the study visit schedule, informed consent from the patient or from

a parent or guardian in the case of children (Teka et al., 2008; WHO, 2009).

Participants who were infected with vivax malaria requiring hospitalization, or had severe malnutrition, febrile condition due to diseases other than malaria, regular medication which might interfere with anti-malarial pharmacokinetics, were pregnant and breastfeeding, were excluded from the study (WHO, 2009).

To our knowledge, there was no earlier report of CRPv in the study area and the sample size was calculated based on the expected proportion of *P. vivax* treatment failure with CQ in the study population. The sample size was calculated with an expected treatment failure rate of 5%, a confidence level of 95% and precision level of 5% using the formula, $N = (Z/d)2\,P\,(1 − P)$ and with 20% expected loss to follow-up over 28 days (WHO, 2009). Therefore, the total sample size for this study was 87 individuals.

2.2. Study design

The study was a single-arm prospective evaluation of clinical and parasitological responses to directly observed treatment of *P. vivax* malaria. People with *P. vivax* mono infection who met the study inclusion criteria were enrolled, treated with CQ and monitored for 28 days. The follow up included a fixed schedule (1, 2, 3, 7, 14, 21, and 28 days) of check-up visits and corresponding clinical and laboratory examinations. Based on the results of these assessments, the patients were classified as having therapeutic failure (early or late) or an adequate response. The proportion of patients with therapeutic failure during the follow-up period was used to estimate the efficacy of CQ (WHO, 2009).

Questionnaire was used to gather socio-demographic information from study participants by senior laboratory technologists. Clinical and laboratory information were also collected as follows.

2.3. Patient recruitment

Patients who met all the inclusion criteria were given a personal identification number and received treatment only after the study was fully explained and informed consent provided. Patients who decided to participate in the study were examined, treated and followed. Successive monitoring of parasitological and clinical responses was made on the follow-up days to each patient until day 28. The day a patient was enrolled and received the first dose of CQ was designated as day 0. Patients were informed to come for follow-up on days 1, 2, 3, 7, 14, 21, and 28. Thick and thin blood smears were prepared and examined for checking parasite clearance and/or recurrence of parasitaemia at all follow-up visits. Hb was measured was on days 0 and 28 during follow-up and on day of recurrence of parasitaemia. Any patient who did not come to the Health Care Centre on the day of appointment was traced at his/her home by the health extension workers to complete the follow-up (Assefa et al., 2015).

2.4. Treatment and follow-up

A 28-day *in vivo*, CQ efficacy testing was done according to methods recommended by WHO (2001) and the Ethiopian Ministry of Health (FMoH, 2006). Patients were treated with a 25 mg/kg CQ-phosphate (Cipla LTD India), administered for three consecutive days (10 mg/kg, 10 mg/kg and 5 mg/kg on days 0, 1 and 2, respectively) (DACA, 2010) at Shawa Robit Health Care Centre. All doses were administered under direct observation. Study subjects were checked for vomiting for 30 min after intake of the drug; those who vomited were retreated with the same dose. The study participants were advised not to take other drugs, except for patients with axillary temperature >37.5 °C who were treated with paracetamol (10 mg/kg). Patients were told to return for follow-up on days 1, 2, 3, 7, 14, 21 and 28. Patients were also advised to come

back to the Health Care Centre if they felt ill at any time during the follow-up period for clinical and parasitological examination.

2.5. Clinical procedures

Physical examinations such as the axillary temperature and clinical conditions were done during the study period for all study participants.

2.6. Laboratory procedure

Capillary blood was collected from each study participant; duplicate thick and thin blood films were made at recruitment and on each follow-up day. The blood films were stained with stained with 10% Giemsa for 10 min, examined with 100× oil immersion objective. Species identification and parasite quantification was done by trained senior medical laboratory technologist and reports were recorded on the laboratory request. The thick blood smears were used to count the numbers of asexual parasites and white blood cells (WBCs) in a limited number of microscopic fields. *P. vivax* asexual stages were counted against 200 WBCs. Parasitaemia was determined using the following formula (WHO, 2009).

Parasite density (per μl of blood)

$$= \frac{WBC\ (8000) \times \text{Number of asexual parasites counted}}{\text{Number of WBC counted}\ (200)}$$

Haematocrit was measured on days 0 and 28 during follow-up for each study participants and on day of recurrence of parasitaemia. Finger-pricked blood was taken and read by portable using microhematocrit technique. Anemia was defined according to (WHO, 2011) WHO classification.

Urine sample of each female participant was screened for pregnancy by Strip Test (PR China, Expiration 2014/11, Batch number W00121125.2). Finally, positive participants were excluded from the study.

2.6.1. Drug quality analysis

The quality of drug used in the study (chloroquine phosphate 250 mg, batch number KT 1474, Cipla LTD, India), was tested for standard concentration using the standard procedures recommended by united state pharmacopeia at the National Laboratory of Ethiopian Food, Medicine and Health care Administration & Control Authority (EFMHACA). CQ–phosphate tablets contain 93–107% of the labeled amount of Chloroquine phosphate. The result of CQ–phosphate test showed the content to be 105.7% that is in the normal range. Therefore, the batch of drug used was confirmed to fulfill international specification set for the test.

2.7. Study endpoints

The primary endpoint of the study was risk of recurrence by day 28 (Getachew et al., 2015; WHO, 2003). Treatment failures were categorized as early treatment failure (ETF), late parasitological failures (LPF) and late clinical treatment failures (LCTF). ETFs were defined as one of the following: (1) the occurrence of danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia, (2) parasitaemia on day 2 higher than on day 0 (initial day) irrespective of axillary temperature, (3) parasitaemia on day 3 with axillary temperature ≥37.5 °C or (4) parasitaemia on day 3 ≥25% of count on day 0. LCTFs were defined as the occurrence of danger signs or severe malaria in the presence of parasitaemia or the presence of parasitaemia on any day between day 4 and day 28 with axillary temperature ≥37.5 °C (or history of fever) in patients who did not previously meet any of the criteria of early treatment failure. LPFs were defined as the presence of parasitaemia on any day between

day 7 and day 28 with axillary temperature <37.5 °C in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure. Patients with no parasitaemia on day 28, irrespective of axillary temperature, and who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure were defined as adequate clinical and parasitological response (ACPR) (WHO, 2003).

2.8. Statistical analysis

Statistical Package for Social Science (SPSS) version 16 was used for data management and analysis. Data of patients having mixed infection with *P. falciparum*, lost to follow-up and vomiting were excluded from the analysis according to WHO method. The analysis included the proportion of early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), and adequate clinical and parasitological response (ACPR) at day 28. Kaplan–Meier survival estimate was used to evaluate risk of therapeutic failure in study participants during follow-up period. Change in mean Hb level on days 0 and 28 was compared using paired *t*-test. In not normally distributed age data, median value was used to measure the central tendency. The geometric mean parasite count was made using geometric mean was made. In all analysis, *p*-value <0.05 was considered significant (Assefa et al., 2015).

2.9. Ethical consideration

The study was reviewed and approved by the Ethical Review Committee of Jimma University, College of Public Health and Medical Sciences. Permission was obtained from the district Health office and administration of Shawa Robit Health Care Centre. The purpose of the study was explained and written informed consent was obtained from each participant and parents or guardians of children.

2.10. Data quality control

Data collectors were trained by experts from the regional referral laboratory before the actual work. Competency of the data collectors was assessed and selected for the study. Standard operating procedures were strictly followed. Quality of reagents and equipment used in the study was checked prior to and during the study period. All *P. vivax*-positive slides on day of admission, all slides on day of recurrence and 5% of negative slides were picked randomly from slides prepared during follow-up and were re-examined blinded by an experts from the regional referral laboratory (Assefa et al., 2015; Ketema et al., 2011).

3. Results

3.1. Study profile and baseline characteristics

Of the 3076 patients were examined for malaria, 296 (9.6%) of them were positive for malaria by blood film microscopy. From the positive cases, 36% (107/296) were with *P. vivax* mono-infection. Among the 107 enrolled eligible for the study, eleven refused to participate and nine of them were rejected because of they took antimalarial drug before recruitment.

Finally, eighty-seven of the patients with *P. vivax* mono-infection, who met the inclusion criteria set by WHO (2009), were enrolled in the study.

As shown in Table 1, the median age of the study population was 20 years (1–65 years), and the majority (71.3%, *n* = 62) of them were males. Among the study participants (33.3%, *n* = 29) were children <15 years. Duration of their illness (mean ± SD) before enrollment

Table 1
Socio-demographic characteristics of patients enrolled in the *in vivo* therapeutic efficacy study of CQ for *P. vivax* malaria at Shawa Robit Health Care Centre North Eastern Ethiopia, October 2013 to February 2014.

Socio-demographic variables	Total no. (%)
Age (in years)	
Median	20
Range	1–65
Gender	
Male	62 (71.3)
Female	25 (28.7)
Ethnicity	
Amhara	62 (71.3)
Argoba	16 (18.4)
Oromo	9 (10.3)
Occupation	
Government employee	13 (14.9)
Unemployed	2 (2.29)
Student	13 (14.9)
House wife	8 (9.19)
Farmer	47 (54)
Merchant	4(4.59)
Marital status	
Single	28 (46.7)
Married	58 29 (51.7)
Widowed	1

Table 2
Summary of patients' characteristics at baseline at Shawa Robit Health Care Centre North Eastern Ethiopia, October 2013 to February 2014.

Number of patients	87
Haematocrit (%), day 0	
Mean (sd)	35.45(5.1)
Range (min–max)	21–46
Mean (sd) duration of illness (days)	3.99 (2.1)
Temperature (°C), day 0	
Mean (sd)	37.3 (0.5)
Range	36–38
Parasitemia (μl), on day 0	
Mean (geometric)	8724
Range (min–max)	440–29,500

was 3.99 ± 2.11 days. From the total study subjects (87 participants), seven of them were censored during the follow up (lost to follow-up on day 3 two cases, on day 7 two cases and on day 14 three cases). On the other hand, four of them were excluded from the study due to *P. falciparum* infection on day 2 one case, on day 7 two cases and on day 14 one case and re-treated with Artemeter–Lumefantrin. From 76 study subjects who successfully completed the 28 follow-up studies, *P. vivax* recurrence was observed in five study subjects (one on day 7, one on day 14 and three on day 21) summarized in Table 2 and Fig. 2 (Khalil et al., 2002).

3.2. Parasite and fever clearance

History of symptoms such as fever, headache, vomiting, cough, diarrhea and joint pain were observed in some patients at the time of recruitment. Among those, fever accounted for (75.7%), headache (63.1%), vomiting (9.1%), diarrhea (4.6%) and joint pain (9.2%).

From those febrile cases, 50.6% (*n* = 44) had fever at admission with mean axillary temperature ≥37.5 °C, while 72.4% (*n* = 63) of the study participants had history of fever in the last 48 h. The geometric mean parasite count of the study participants at baseline was 8724/μl (95% CI = 440–29,500). It was observed that age and parasite density of the study population at day 0 had negative correlation ($r = -0.355$, significant at $p = 0.01$).
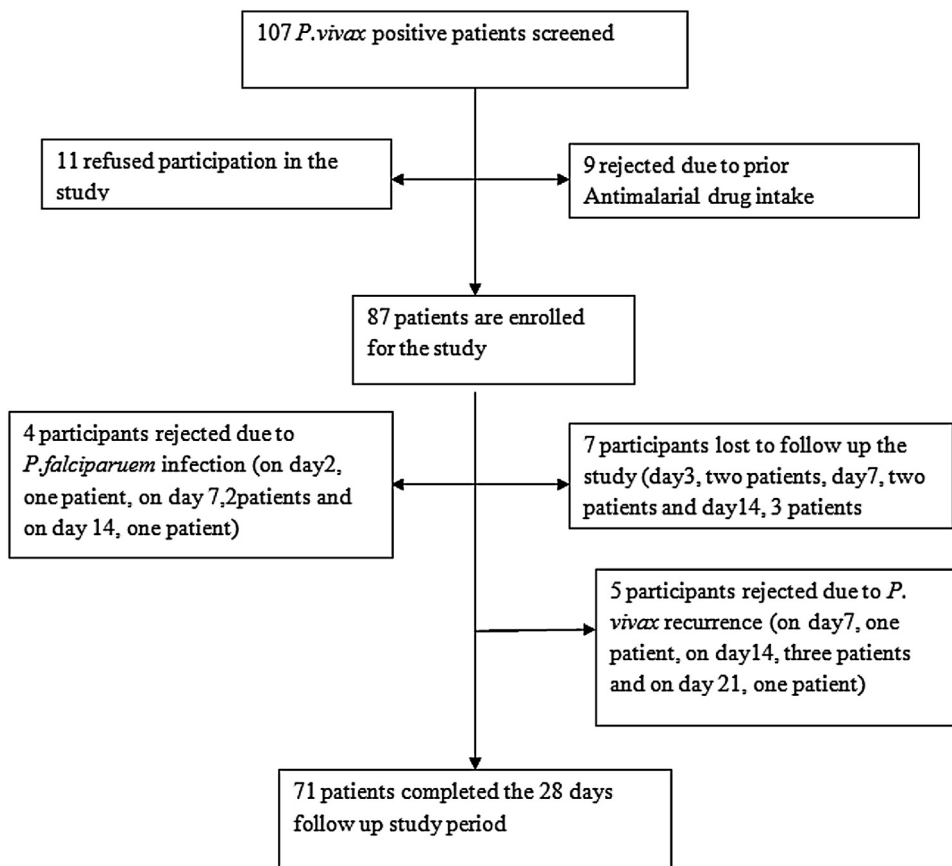
Fig. 2. Flow chart shows patient enrolment for therapeutic efficacy study of chloroquine at Shawa Robit Health Centre Town, North Eastern Ethiopia, and October 2013 to February 2014.

Table 3
Summary of treatment outcome of the study participants in Shawa Robit Town, north eastern Ethiopia, October 2013 to February 2014.

	Number	%	95%CI
ETF	0	0.0	0.0–4.7
LCF	0	0.0	0.0–4.7
LPF	5	6.6	2.2–14.7
ACPR	71	93.4	85.3–97.8
Total patients per protocol	76		
Withdrawn from study	4		
Loss of follow up	7		
Total loss of follow up/withdrawn	11	12.6	
Total patients at baseline	87		
Day 3 parasite clearance			
Day 3 positive	0	0.0	0.0–4.2
Total analysis at day 3	86		

ETF, early treatment failure; LCF, late clinical failure; LPF, late parasitological failure; ACPR, adequate clinical and parasitological response.

Table 4
Regression estimate of predictor variables on prevalence of CQ treatment failure in Shawa Robit Town, North East Ethiopia, October 2013 to February 2014.

	AOR	Lower	Upper	p-Value
Age	0.226	0.021	2.439	0.221
Parasite density	6.221	0.578	66.945	0.132
Income	0.245	0.031	1.936	0.182
Constant	0.118			0.100

Parasite clearance was achieved within 48 h in 81 (93.1%) of the study participants, and full parasite clearance was observed on day 3 in all cases. The mean parasite reduction ratio was 34.14 on day 2. Gametocytes were detected from the blood films of 46 (52.9%) of the study subjects on days of admission. Gametocytaemia was cleared within 3 days and did not re-appear until the end of the follow-up period.

Among the 87 patients treated with CQ, five (6.6%) were with LPF on day 7 (one case), on day 14 (three cases) and on day 21 (one case). Seventy-one (93.4%) participants showed ACPR. There was no ETF or LCF in the present study (Table 3). The cumulative incidence of success and failure rates at day 28 was 93.4% (95% CI = 0.849–0.972) and 6.6% (95% CI = 0.028–0.151), respectively (Fig. 3). In all patients

with treatment failure, parasite count decreased from day of admission to day of parasite recurrence. All of the study participants with treatment failure were without complains of malaria symptoms except for the recurrence of parasitaemia. Four of the patients with treatment failure cleared their parasitaemia within 48 h, while one of the patients with treatment failure cleared parasitaemia on day 3.

3.3. Treatment failure associated factors

Binary logistic regression (Table 4) was performed to identify factors associated with treatment failure. Age, sex, HCT level, asexual parasite density, temperature, residence, income and level of education were used as predictor variables. In the binary logistic regression, only asexual parasite density showed a statistically significant association with treatment failure (OR = 10.261, 95% CI: 1.088–96.736, p-value = 0.042). Variables with p-value of less than 0.25 at preliminary bivariate analysis were selected as candidate for multivariate analysis. Accordingly, age, asexual parasite density and income were found to have a p-value of less than 0.25 in initial bivariate analysis, were selected as candidate variables and
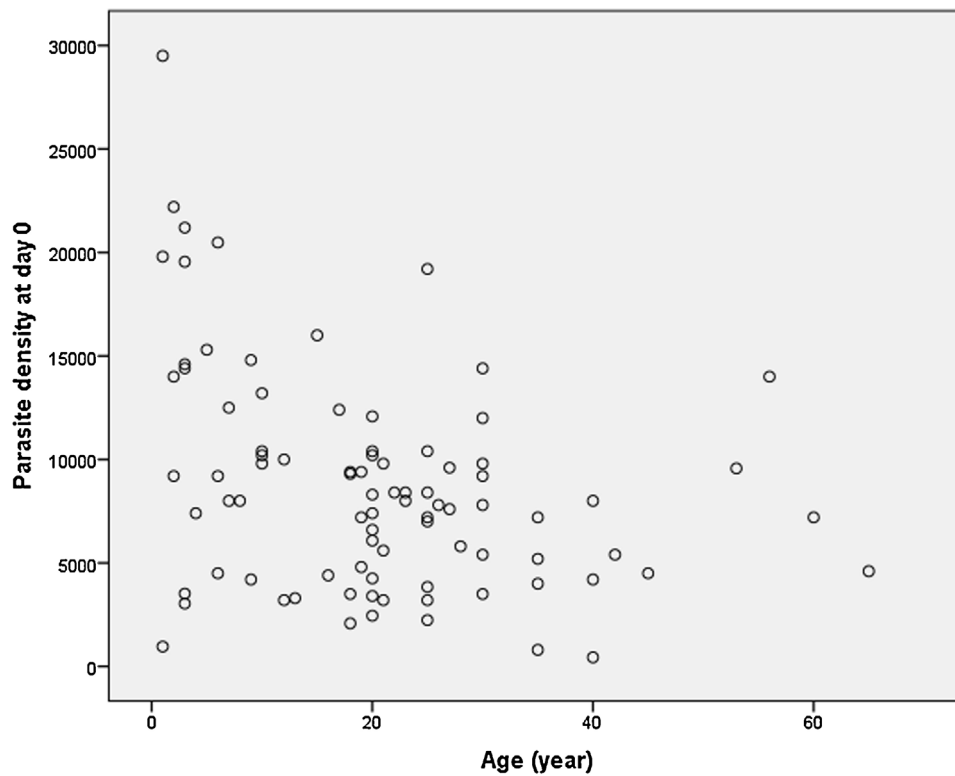
Fig. 3. Kaplan–Meier survival curve of the study in Shawa Robit Town, North Eastern Ethiopia October 2013 to fever Parasite Clearance Times.

entered into multivariate logistic regression analysis. In multivariate logistic regression, age, asexual parasite density and income did not show a statistically significant association with treatment failure (Table 4).

3.4. Haematocrit measurement

The mean haematocrit level of the study participants on day 0 was 35.45% (21–46%) while it was 38.44% on day 28. Moreover, the mean haematocrit level on day of admission and day 28 were significantly different (Wilcoxon Signed Ranks Test, $p = 0$) (Fig. 4).

In addition, chi-square goodness-of-fit test (Pearson's chi-square goodness-of-fit test) was used to determine the proportion of anemic cases on day of admission and on day 28. The proportion of anemic cases on day of admission was not significantly different ($\chi^2 = 0.56$, $p = 0.45$). However, on day 28, the number of anemic cases differed significantly ($\chi^2 = 39.56$, $p = 0.00$). Similarly, in two of the study participants with treatment failure the level of HCT decreased on day of recurrence compared to the day of admission. On the contrary, in three of the patients with treatment failure there was no change in the HCT level on day of enrolment and on day of recurrence (Fig. 5).

4. Discussion

Studies conducted in various parts of elsewhere in Africa revealed that the rate of treatment failure ranges from 0% to 84% was reported (Fryauff et al., 1998; Soto et al., 2001; Trape et al., 1998). In Ethiopia, CQ is the recommended anti-malaria drug for treatment of *P. vivax* malaria infection. However, reports of emergence of CRPv are appearing in some parts of the country (Ketema et al., 2011; Teka et al., 2008; Tulu et al., 1996). The current study showed no early treatment failure and other treatment failures observed on days between 7 and 28. This is in contrary to previous study done in southern Ethiopia in which two (0.7%) patients expe-

rienced early treatment failure and 23 (8%) late treatment failure (Getachew et al., 2015). The days of recurrence in our study were on day 7 (one case), day 14 (three cases) and day 21 (one case). This finding is however different from the two study reported from Debre-Zeit, where the day of recurrence for all the study participants was day 28 (Teka et al., 2008). The difference on the day of recurrence could be due to the presence of variation in the degree of resistance in different parts of the country.

In support of the previous reports elsewhere, the current study also confirmed CQ treatment failure for vivax malaria in Shawa Robit. Hence, our finding unveils late LPF rate of 6.6% ($n = 5$). This finding is comparable with the previous reports in Ethiopia from (Teka et al., 2008) and Serbo Town (Ketema et al., 2009) in which treatment failure rates were 4.6% and 3.6% respectively. Comparable results were also reported with treatment failure from Madagascar (5.1%) (Barnadas et al., 2008).

Five of the study subjects with LPF were without complains of malaria symptom except for the recurrence of parasitaemia. This finding is comparable with the study finding in Serbo Town, in which none of the patients with treatment failure had complained of malaria symptom (Ketema et al., 2009). However, our finding is different from a study finding in Debre Zeit, in which four of the patients with recurrent parasitaemia were symptomatic (Teka et al., 2008).

Because most of the treatment failures detected in this study were at the early stage (before Day 17) of the follow up (Day 7, and 14, except one case on day 21) on these days the blood level of CQ and DCQ do not drop to level below the MEC (Baird, 2004). Accordingly, the concentration of the drug at this time can prevent the recurrence of parasitemia of relapse or re-infection. Even though, CQ blood concentration data at the day of treatment failure was not available to confirm resistance (which we did not perform), the patients were treated with a standard 25 mg/kg CQ regimen and all drug doses were administered under direct observation by the research group and none of the study subjects had recurrent vomit-
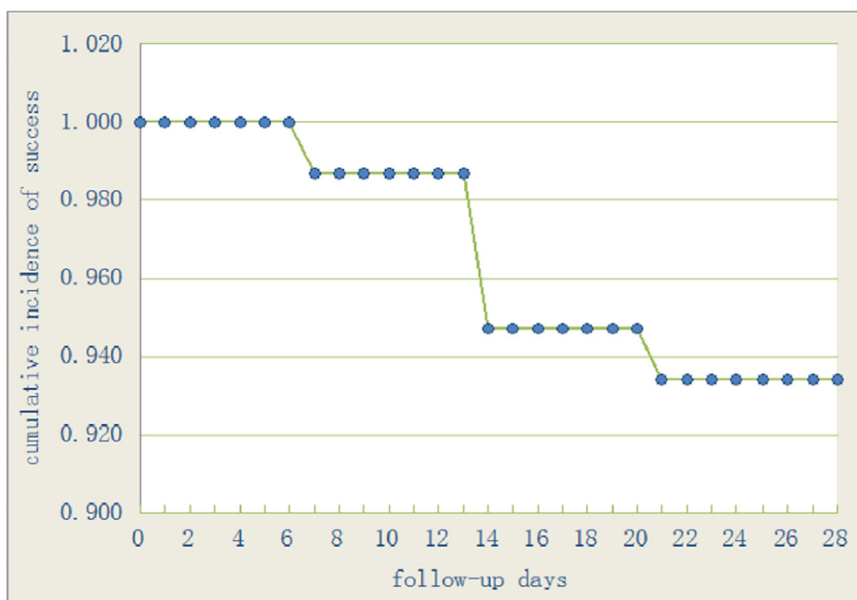
Fig. 4. Kaplan–Meier survival curve of the study in Shawa Robit Town, North East Ethiopia, October 2013 to February 2014.
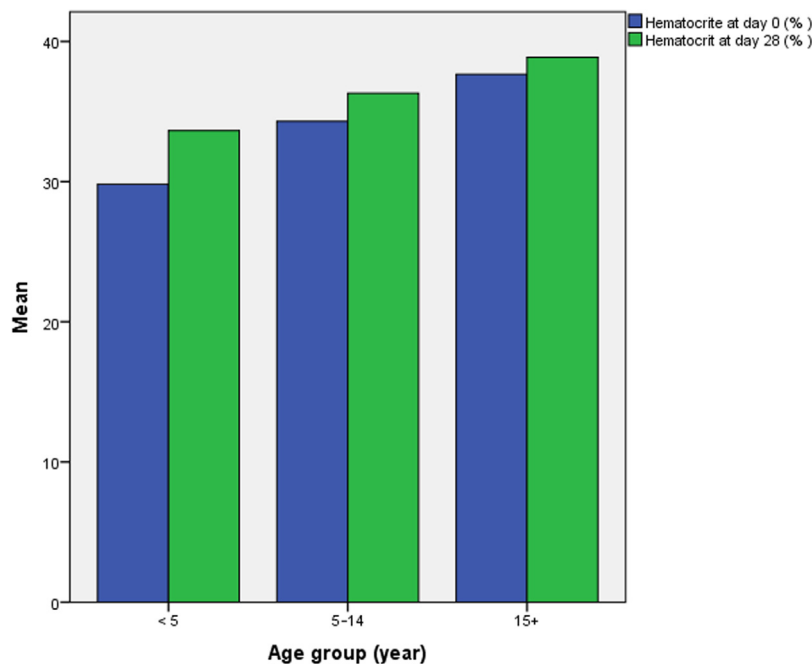

Fig. 5. Hematocrit recoveries among study participants with adequate clinical and parasitological response (n = 71) in different age groups in ShoaRobit town, North East Ethiopia, October 2013 to February 2014.

ing. These situations were expected to reduce the risk of treatment failure attributable to poor dosing.

The study participants with treatment failure were children at age of 1, 3, 8, 9 and 17 years. This is in agreement with earlier reports elsewhere in Ethiopia in which treatment failures were observed in children (Ketema et al., 2009, 2011; Teka et al., 2008).

Findings from this study depicted that higher parasitaemia on day 0 was not statistically associated with treatment failure. The load of parasitaemia on the day of treatment failure was lower than the day of admission. This is similar with the study done in Halaba district, South Ethiopia (Ketema et al., 2011; Teka et al., 2008) and different from the studies conducted in Colombia and Serbo Town in South-West Ethiopia (Ketema et al., 2009; Soto et al., 2001).

In this study, the same batch of standard CQ was used. All the study participants were given a directly observed treatment (DOT) and none of them vomited the drug. None of the study participants responded to take antimalarial drugs before enrollment.

Therefore, as limitation of the current study, poor adherence, the blood levels of the drug and genotyping to distinguish heterologous from homologous infections were not studied.

In conclusion, the current study showed that there is a 6.6% LPF rate of CQ treatment failure, indicating possible emergence of CRPv strains in the study area.

Finally, CQRv molecular markers and regular monitoring of the pattern of resistance to CQ is needed for rapid and effective control measures of possible spread of drug resistance in the study area.

Authors' contributions

SS, AB, EZ, SS and AZ conceived and designed the study. SS involved in data acquisition. AZ supervised the data collection. SS, AB, EZ and AZ involved in data analysis and interpretation. SS and AB drafted the manuscript. All authors read and approved the submitted version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2017.02.027.

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